Exhibit A

PATENT APPLICATION
FILING ACKNOWLEDGMENT
PROVISIONAL

Mailing Date: March 22, 2000
File No.: 014058-005640US Attorney: KEVIN BASTIAN
Inventor(s): CARTER, Darrick
Title: Immune Mediators and Related Methods

_____ Declaration _____ Power of Attorney
_____ Combined Declaration & Power of Attorney
_____ Assignment _____ Small Entity Decl.

jc710 U.S. PTO
60/191274
03/22/00

No. Pages of Description: 43
No. Pages of Appendix: _____
No. Pages of Claims: _____
No. Pages of Abstract: 1
No. Sheets of Drawings: _____
Microfiche Appendix: _____

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CARTER	Darrick		

Title: IMMUNE MEDIATORS AND RELATED METHODS

Enclosed are:
[X] 43 pages of the specification (including description and claims).
[X] 1 page of Abstract.
[ ] The invention was made by or under a contract with the following agency of the United States Government: _____ under Government contract number _____

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PROVISIONAL PATENT APPLICATION
IMMUNE MEDIATORS AND RELATED METHODS

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IMMUNE MEDIATORS AND RELATED METHODS

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application is related to U.S. Serial No. 09/261811, filed March 3, 1999, which is a continuation-in-part of U.S. Serial No. 08/480,002, filed June 7, 1995, now abandoned, U.S. Serial No 08/657,581 filed June 7, 1996, now abandoned and U.S. Serial No 09/184,692 November 2, 1998, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

There is currently a great interest in developing pharmaceuticals based on the growing understanding of the structure and function of the major histocompatibility complex (MHC) antigens. These cell surface glycoproteins are known to play an important role in antigen presentation and in eliciting a variety of T cell responses to antigens. Identification of synthetic antigenic peptides, and demonstration that these peptides bind selectively to MHC molecules associated with disease and that stimulates T cells would help to implicate a particular peptide or peptide:MHC complex in susceptibility to an autoimmune disease. In particular, the development of soluble, fused MHC heterodimer:peptide complexes would be particularly useful in treatment of a number of diseases associated with antigen presentation by MHC molecules. The present invention fulfills such needs, and provides related advantages.

SUMMARY OF THE INVENTION

The present invention provides recombinant DNA constructs that encode soluble, fused MHC heterodimers that may or may not be further linked to an antigenic peptide. Typically, the constructs comprise a first DNA segment encoding at least a portion of a first domain of a selected MHC molecule; a second DNA segment encoding at least a portion of a second domain of the selected MHC molecule; a first linker DNA segment and connecting in-frame the first and second DNA segments; wherein linkage of the first DNA segment to the second DNA segment by the first linker DNA segment results in a fused first DNA-first linker-second DNA polysegment. The constructs of the invention may also comprise a third DNA segment encoding an antigenic peptide capable

of associating with a peptide binding groove of the selected MHC molecule and a second linker DNA segment connecting in-frame the third DNA segment to the fused first DNA-first linker-second DNA polysegment. The linkage of the third DNA segment to the fused first DNA-first linker-second DNA polysegment by the second linker DNA segment results in a soluble, fused MHC heterodimer:peptide complex.

As shown below, the invention provides preferred constructs, comprising for example preferred linker sequences. The linkers and other sequences used in the constructs of the invention are particularly useful in producing complexes that fold properly and have enhanced therapeutic activity.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Prior to setting forth the invention, it may be helpful to an understanding thereof to provide definitions of certain terms to be used hereinafter:

Fused MHC heterodimer:peptide complex (also referred to as half-anergix): As used herein it refers to a fusion protein such as the fused, MHC heterodimer:peptide complex of the invention. Such fusion proteins will be indicated with a colon (:). MHC-peptide complexes which are not, fusion proteins, are native MHC containing protein or exogenously loaded MHC molecules are indicated with a dash (-).

A domain of a selected MHC molecule: A portion of an MHC domain which is sufficient to form, either alone, or in combination with another portion of an MHC domain, a peptide binding site which is capable of presenting an antigentic peptide in such a fashion that it is recognized by a T cell receptor. Such MHC domains would include the extracellular portion of the two polypeptide chains of either Class I or Class II MHC. This would include any or all of the domains of α chain (α1, α2, or α 2) and β2-microgloublin subunit of Class I MHC. For example, Class I MHC domains would include any combination of the three α chain domains either independent of the others, α1, α 2, or α3, in tandem, α1α2, α2α3, α1α3, and/or the β2 domain. Also included are the α chain (α1, α2) and β chain (β1, β2) of Class II MHC. This would include α1 or α2 independent of the other, or α1 and α2 in tandem (α1α2). It would also include β1 or β2 independent of the other, or β1 and β2 in tandem (β1β2).

Linker DNA segment: A segment of DNA (typically encoding about 5 to about 25 amino acids), prototypically repeating glycine residues with interspersed serine residues which forms a flexible link between two DNA segments. This flexible link

allows the two DNA segments to attain a proper configuration, such as an MHC peptide binding groove, or allows a peptide to properly bind into such a groove.

Antigenic peptide: A peptide which contains an epitope recognized by immune cells, particularly T cells, and is capable of stimulating an MHC-mediated immune response.

The complexes of the invention can be made according to standard recombinant techniques described, for example in the above referenced related applications, WO 96/40944 and in US Patent No. 5, 869,270. These patent documents also provide description of how to use the complexes to treat diseases, for example, autoimmune diseases.

The following examples are offered by way of illustration, not by way of limitation.

EXAMPLES

EXAMPLE 1

Construction of DNA sequences encoding human soluble, fused MHC heterodimer:peptide complexes, HLA-DR4 molecules(CO563 or CO564).

DNA constructs encoding gp39 β1/α1 human molecules, were prepared from a cDNA encoding the gp39 peptide fused to the β1/α1 domains of HLA-DR4 according to standard techniques.

For the production of "empty" β1/α1 DR4 molecules, a cDNA encoding the fused β1/α1 domains of HLA-DR4 was prepared using cloned α and β chains from DR4.

Amino acid sequence of the gp39-β1/α1 HLA-DR4 human half anergix molecule (linkers are shown in bold)

MGDTGRSFTLASSETGVG**ASGGGGSGGGG**DTRPRFLEQVKHECH FFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQ KRAAVDTYCRHNYGVGESFTVQRRGGIKEEHVIIQAEFYLNPDQSGEFMFDFDG DEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPIT N*

aa1 – aa4 : leader sequence

aa5 - aa18 : gp39 peptide

aa19 - aa28 : linker

aa29 - aa122 : HLA-DR4 β1 domain

aa123 – aa124 : linker

aa125 – aa 208: HLA-DR4 α1 domain

Amino acid sequence of the " empty " β1/α1 HLA-DR4 human half anergix molecule

MGDTRPRFLEQVKHECHFFNGTERVRFLDRYFYHQEEYVRFDSDV
GEYRAVTELGRPDAEYWNSQRDLLEQKRAAVDTYCRHNYGVGESFTVQRRGGI
KEEHVIIQAEFYLNPDQSGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEA
QGALANIAVDKANLEIMTKRSNYTPITN*

aa 1 – aa95: HLA-DR4 β1 domain

aa96- aa97 : linker

aa98- aa181 : HLA-DR4 α1 domain

3- Production and purification of half anergix molecules.

For the production of recombinant proteins, the bacteria (pLysS) were grown in LB (containing ampicilline (50 µg / ml) and chloramphenicol (5 µg / ml)) at 37°C until OD600 = 0.5. IPTG was added at the final concentration of 0.5 mM final and the bacteria were further incubated for 3 hours at 37°C with shaking. The bacteria were centifuged at 4°C, 4K for 20 min and the pellet was frozen at –80°C. The following day, the pellet was resuspended in 40 ml of lysis buffer (50 mM Tris-HCl pH8, 50 mM NaCl, 2 mM EDTA, 1 protease inhibitor cocktail tablet, 1% Triton X100 and 1% deoxycholate), and incubated for 1 hour at 4°C under continuous agitation with a magnetic stirrer. The sample was then homogenized using a French Press with a 1000 psi setting, and centrifuged at 4°C, 9000g for 20 min. The pellet was then resuspended in 30 ml of lysis buffer without Triton and deoxycholate and centrifuged at 4°C, 9000 g for 20 min. The new pellet was resuspended in 10 ml of 20 mM ethanolamine/6 M urea pH10, and eventually frozen at –80°C. The recombinant protein was then purified by FPLC ion-exchange chromatography using Source 30Q anion-exchange media in an XK26/20 column using a step gradient going from 1 mM to 1 M NaCl in 20 mM ethanolamine/6M urea pH10. Fractions were analysed by SDS/PAGE and those corresponding to the proteins of interest are pooled and dialysed against PBS 1X.

EXAMPLE 2

Production of Additional Constructs

Additional constructs with different composition and length of the 2nd linker (between β1 and α1) were engineered by using standard techniques using CO567 as the template. Specifically, PCR primers were designed to replace the old sequence in CO567 with the new sequence. For example to make CO581, the primers were designed with the following sequences (note these primers were phosphorylated at 5').

Primer 1: 5' pCACCAGGAGGAGAGCCGCCCACGCCGGTCTCGCTGG

Primer 2: 5' pGACCACCTGGATCTGGGGACACCCGACCACGTTTC

PCR reaction (100 ul) was made of the following components: 2 μl CO567 (80 ng) as template, 2 μl each of primer 1 and primer 2 (10 μM), 2 μl of dNTP mix (20 mM each), 10 μl of 10X pfu buffer, and 80 μl of sterile water. After all the components were mixed, 2 μl of Turbo pfu (5U total) was added, mixed and put on PCR machine. The PCR cycles has a pre-denaturation at 95C for 30 sec, then 10 cycles of 95C for 30 C, 60C r 1 min, and 72C for 7 min. Then another 22 cycles of 95C for 30C, 65C for 1 min and 72C for 7 min, followed by a final 10 min at 72C.

The PCR mixture was digested with 2 μl of dpn I (10U) for 2h at 37C. Then the PCR product at ~6 kb was purified from agarose gel after electrophoresis. The purified PCR product was ligated by T4 DNA ligase for 1 h at room temperature then used to transform into NovaBlue (Novagen)competent cells by standard protocol. Cells were plated on LB (+Carb) and grow overnight at 37C.

Next day, about a dozen single colony from the transformation were randomly picked for overnight culture in 5 ml LB (+Carb) at 37 C. Plasmids from these culture were purified with Wizard Miniprep kit, and analyzed by Xho I digestion. A few plasmids that passed the Xho I digestion were further confirmed by DNA sequencing.

To express the recombinant proteins, a clone with confirmed DNA sequence was used to transform BL21(DE3)CodonPlus-RIL (Stratagene) by standard transformation protocol and plated on LB (Carb+Cam) plates overnight at 37C. Next

morning, a single colony was picked to inoculate 100 ml LB (+Carb +Cam) and the culture was grown till OD reach between 0.8-1.0, and stored overnight at 4C. Next day, the culture was pellet down and used to inoculate into 2xYT (+Carb+Cam) at ratio of 25 ml culture per liter new media. These large cultures were grown at 37C till OD=0.5-0.6, and IPTG was added to induce recombinant protein at 37C for 3 h. The induced cultures were pellet and stored at –80C till purification.

Table 1 provides a listing of various constructs made according to the invention.

Construct	peptide	upstream linker	downstream linker
C0523	yes	GGGG	GG
C0543	none	none	GG
C0563	yes	ASGGGSGGG	GG
C0567	yes	ASGGGSGGG	TSGGGGSGGGGSSS
C0580	yes	ASGGGSGGG	GSPGGGGSGGGPGS
C0581	yes	ASGGGSGGG	GSPPGGPPGS
C0582	yes	ASGGGSGGG	GSPGGGGPGS
C0583	yes	ASGGGSGGG	TSGGGGS
C0584	yes	ASGGGSGGG	SGGSGGS
C0585	yes	ASGGGSGGG	FDAPSPLP
C0586	none	none	TSGGGGSGGGGSSS
C0587	none	none	GSPGGGGSGGGPGS
C0588	none	none	GSPPGGPPGS
C0589	none	none	GSPGGGGPGS
C0590	none	none	TSGGGGS
C0591	none	none	SGGSGGS
C0592	none	none	FDAPSPLP
CO593	yes	ASGGGSGGG	VYPEVTV
CO594	none	none	VYPEVTV
CO595	yes	ASGGGSGGG	GGGG
CO596	yes	ASGGGSGGG	GGGGS
CO597	yes	ASGGGSGGG	GGGSGG

Table 1

EXAMPLE 3

The need for novel linker sequences was realized after inspection of circular dichroism spectra of purified, refolded ½ Anergix constructs. Inspection of the atomic structures of the various murine and human MHC class II molecules as determined by X-ray crystallography indicated that these molecules have a high degree of structural similarity. The circular dichroism results were consistent with two folded molecules of clearly different secondary structure. Careful inspection of the structures reveals that the human MHC has a longer distance between chains than the equivalent murine molecule. This led to proposing longer linkers between the chains which would contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). These constructs make up the first class of linkers. To inhibit the continuation of secondary structure across the linker, prolines were added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. These linkers make up the second class of linkers dislcosed here.

Next, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. These are the third class of linkers. Finally, a combination of these types of linkers could also be used. These are the fourth class of linkers.

EXAMPLE 4

As it was known that the murine ½ Anergix construct is functional, another linker has been suggested based on a fusion of the two molecules. This fusion would incorporate residues from the functional murine ½ Anergix as a linker and the alpha and beta chains of the human molecule would be used to give the desired function. The protein sequence of the molecule to be expressed is below:

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECHFFNGTERVRF
LDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQKRAAVDTYC
RHNYGVGESFTV**LRRLGGEDDEADH**HVIIQAEFYLNPDQSGEFMFDFDGDEIFH
VDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITN*

The linker residues from the mouse construct are in bold.

EXAMPLE 5

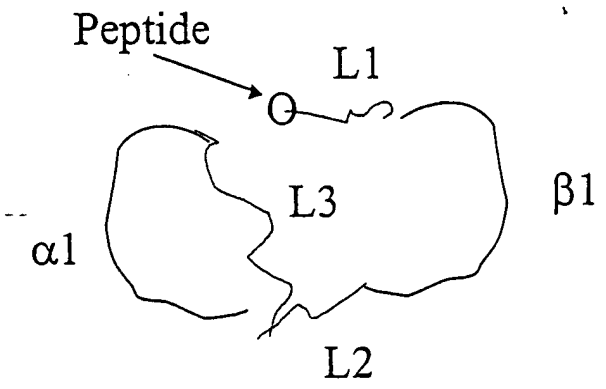
Further MHC Class II hybrid molecules may be design by fusing other portions of the alpha chain and the beta chain together using linkers as described

elsewhere. A properly folded molecule may be obtained by putting appropriate linkers between portions of the human MHC class II which are proximal to each other as determined by visual inspection of the atomic coordinates of residues of the native MHC available in the publicly accessible protein structure database. These structures would predict possible fusion proteins which covalently attach any part of the beta chain between residues 82 to 123 or between residues 148 to 164 to portions of the alpha chain such as the N-terminal residues, residues 79 to 84, or 92 to 106. Which combination of linker / domain from alpha chain / domain from beta chain is preferable remains to be determined by producing combinatorial fusions between these areas. The numbering system of residues in this example corresponds to those found in the coordinates of the structure described in: DESSEN, *et al. Immunity* 7:473 (1997). Other, homologous residues could be used to create equivalent constructs for genotypic and allelic variants of these molecules e.g. equivalent residues in DR2 or such. DNAs for such hybrids would be prepared and expressed in a recombinant expression system by someone skilled in the art and could be assayed for structure and function in appropriate assays.

EXAMPLE 6

Use of CD4 binding site(s) of MHC class II molecules as linkers for the production of bioactive recombinant MHC peptide truncated molecules

HLA class II molecules present antigenic peptides to the T cell receptor of the CD4+ T lymphocytes and interact with CD4 during the antigen recognition process. Native MHC class II-peptide complexes have been shown to bind to MHC class II restricted and antigen specific TCRs on a particular T cell and induce T cell nonresponsiveness. It is proposed that the CD4 binding site is important in the docking of MHC class II-Peptide complex with the TCR and induce nonresponsiveness. Since the binding of CD4 to MHC class II-peptide is important in antigen presentation and/or induction of T cell nonresponsiveness, we propose that recombinant MHC class II-peptide molecules (truncated or whole) containing CD4 binding site will be biologically active. We further propose that a polypeptide fragment from MHC class II which binds the CD4 if used as a linker in preparation of MHC class II-peptide truncated molecules the resulting recombinant molecules will be biologically active.



The above Figure shows the concept of different linkers. Peptide is attached to a linker 1 (L1) which is attached to N-terminus of β1 domain that is linked to L2. L2 is linked to L3, which in turn is linked to N-terminus of α1 chain of MHC class II. Here L2 represents the human CD4 binding sequences. It should be noted that L2 could also be directly linked to N-terminus of α1 domain by completely deleting L3.

Specific examples of L1and L3 are given in a examples.

The sequences of L2 are given below. These sequences are applicable to most of the DR-Peptide molecules.

1. **RNGQEEKAGVVSTGLI**
2. **RNGQETKAGVVSTGLI**
3. **YNQQEEKAGGVSTGLI**
4. **FRNGQEEKAGVVSTGLI**
5. **FRNGQETKAGVVSTGLI**
6. **FYNQQEEKAGGVSTGLI**
7. **LNGQEEKAGMVSTGLI**

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the

invention. Accordingly, the invention is not limited except as by the appended claims. All patents, patent applications, and publications are incorporated herein by reference.

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tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240
agacacaact acggggttgg tgagagcttc acagtgcagc ggcgaactag tggtggcggt 300
ggcagcatca aagaagaaca tgtgatcatc caggccgagt tctatctgaa tcctgaccaa 360
tcaggcgagt ttatgtttga ctttgatggt gatgagattt tccatgtgga tatggcaaag 420
aaggagacgg tctggcggct tgaagaattt ggacgatttg ccagctttga ggctcaaggt 480
gcattggcca acatagctgt ggacaaagcc aacctggaaa tcatgacaaa gcgctccaac 540
tatactccga tcaccaatta a 561

<210> 9 CO589
<211> 570
<212> DNA
<213> Homo sapiens

<400> 9
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gggacggagc gggtgcggtt cctggacaga tacttctatc accaagagga gtacgtgcgc 120
ttcgacagcg acgtggggga gtaccgggcg gtgacggagc tggggcggcc tgatgccgag 180

tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240
agacacaact acggggttgg tgagagcttc acagtgcagc ggcgaggctc tcctggaggt 300
ggaggtcctg gatctatcaa agaagaacat gtgatcatcc aggccgagtt ctatctgaat 360
cctgaccaat caggcgagtt tatgtttgac tttgatggtg atgagatttt ccatgtggat 420
atggcaaaga aggagacggt ctggcggctt gaagaatttg gacgatttgc cagctttgag 480
gctcaaggtg cattggccaa catagctgtg gacaaagcca acctggaaat catgacaaag 540
cgctccaact atactccgat caccaattaa 570

<210> 10 CO588
<211> 570
<212> DNA
<213> Homo sapiens

<400> 10
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gggacggagc gggtgcggtt cctggacaga tacttctatc accaagagga gtacgtgcgc 120
ttcgacagcg acgtgggggga gtaccgggcg gtgacggagc tggggcggcc tgatgccgag 180
tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240
agacacaact acggggttgg tgagagcttc acagtgcagc ggcgaggctc tcctcctggt 300
ggaccacctg gatctatcaa agaagaacat gtgatcatcc aggccgagtt ctatctgaat 360
cctgaccaat caggcgagtt tatgtttgac tttgatggtg atgagatttt ccatgtggat 420
atggcaaaga aggagacggt ctggcggctt gaagaatttg gacgatttgc cagctttgag 480
gctcaaggtg cattggccaa catagctgtg gacaaagcca acctggaaat catgacaaag 540
cgctccaact atactccgat caccaattaa 570

<210> 11 CO587
<211> 582
<212> DNA
<213> Homo sapiens

<400> 11
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gggacggagc gggtgcggtt cctggacaga tacttctatc accaagagga gtacgtgcgc 120
ttcgacagcg acgtgggggga gtaccgggcg gtgacggagc tggggcggcc tgatgccgag 180
tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240
agacacaact acggggttgg tgagagcttc acagtgcagc ggcgagggag tccaggcggt 300
ggcgggagcg gcggtggtcc tgggagtatc aaagaagaac atgtgatcat ccaggccgag 360
ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 420
ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 480
gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 540
atcatgacaa agcgctccaa ctatactccg atcaccaatt aa 582

<210> 12 CO586
<211> 582
<212> DNA
<213> Homo sapiens

<400> 12
atgggggaca cccgaccacg tttcttggag caggttaaac atgagtgtca tttcttcaac 60
gggacggagc gggtgcggtt cctggacaga tacttctatc accaagagga gtacgtgcgc 120

ttcgacagcg acgtggggga gtaccgggcg gtgacggagc tggggcggcc tgatgccgag 180
tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240
agacacaact acggggttgg tgagagcttc acagtgcagc ggcgaactag tggtggcggt 300
ggcagcggcg gtggtggttc ctcgagtatc aaagaagaac atgtgatcat ccaggccgag 360
ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 420
ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 480
gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 540
atcatgacaa agcgctccaa ctatactccg atcaccaatt aa 582

<210> 13 CO602
<211> 186
<212> PRT
<213> Homo sapiens

<400> 13
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu
85 90 95

Gly Gly Glu Asp Asp Glu Ala Asp His His Val Ile Ile Gln Ala Glu
100 105 110

Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp
115 120 125

Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp
130 135 140

Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala
145 150 155 160

Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys
165 170 175

Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> 14 CO601
<211> 186
<212> PRT
<213> Homo sapiens

<400> 14
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Gly Gly Ser Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu
100 105 110

Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp
115 120 125

Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp
130 135 140

Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala
145 150 155 160

Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys
165 170 175

Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> 15 CO600
<211> 185
<212> PRT
<213> Homo sapiens

<400> 15
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe
100 105 110

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly
115 120 125

Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg
130 135 140

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu
145 150 155 160

Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg
165 170 175

Ser Asn Tyr Thr Pro Ile Thr Asn
180

<210> 16 CO599
<211> 184
<212> PRT
<213> Homo sapiens

<400> 16
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Gly Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr
100 105 110

Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp
115 120 125

Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu
130 135 140

Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala
145 150 155 160

Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser
165 170 175

Asn Tyr Thr Pro Ile Thr Asn
180

<210> 17 CO594
<211> 187
<212> PRT
<213> Homo sapiens

<400> 17
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys

65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val
85 90 95

Tyr Pro Glu Val Thr Val Ile Lys Glu Glu His Val Ile Ile Gln Ala
100 105 110

Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe
115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val
130 135 140

Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly
145 150 155 160

Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr
165 170 175

Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> 18 CO592
<211> 188
<212> PRT
<213> Homo sapiens

<400> 18
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe
85 90 95

Asp Ala Pro Ser Pro Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln
100 105 110

Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp
115 120 125

Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr
130 135 140

Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln
145 150 155 160

Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met
165 170 175

Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> 19 CO591
<211> 187
<212> PRT
<213> Homo sapiens

<400> 19
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser
85 90 95

Gly Gly Ser Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala
100 105 110

Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe
115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val
130 135 140

Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly
145 150 155 160

Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr
165 170 175

Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> 20 CO590
<211> 187
<212> PRT
<213> Homo sapiens

<400> 20
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr
85 90 95

Ser Gly Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala
100 105 110

Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe
115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val
130 135 140

Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly
145 150 155 160

Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr
165 170 175

Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn

180 185

<210> 21 CO589
<211> 190
<212> PRT
<213> Homo sapiens

<400> 21
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Ser Pro Gly Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile
100 105 110

Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met
115 120 125

Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys
130 135 140

Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu
145 150 155 160

Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu
165 170 175

Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> 22 CO588
<211> 190
<212> PRT
<213> Homo sapiens

<400> 22
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Ser Pro Pro Gly Gly Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile
100 105 110

Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met
115 120 125

Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys
130 135 140

Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu
145 150 155 160

Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu
165 170 175

Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> 23 CO587
<211> 194
<212> PRT
<213> Homo sapiens

<400> 23
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Ser Pro Gly Gly Gly Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu
100 105 110

Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser
115 120 125

Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp
130 135 140

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe
145 150 155 160

Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys
165 170 175

Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr
180 185 190

Asn

<210> 24 CO586
<211> 194
<212> PRT
<213> Homo sapiens

<400> 24
Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr
85 90 95

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu
100 105 110

Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser
115 120 125

Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp
130 135 140

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe
145 150 155 160

Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys
165 170 175

Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr
180 185 190

Asn

<210> 25 CO598
<211> 639
<212> DNA
<213> Homo sapiens

<400> 25
atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgctg 360
cgccgactcg gaggtgaaga tgacgaggca gatcaccatg tgatcatcca ggccgagttc 420
tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatggtga tgagattttc 480
catgtggata tggcaaagaa ggagacggtc tggcggcttg aagaatttgg acgatttgcc 540
agctttgagg ctcaaggtgc attggccaac atagctgtgg acaaagccaa cctggaaatc 600
atgacaaagc gctccaacta tactccgatc accaattaa 639

<210> 26 CO597

<211> 639
<212> DNA
<213> Homo sapiens

<400> 26
atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggag gtggaagcgg cggaatcaaa gaagaacatg tgatcatcca ggccgagttc 420
tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatggtga tgagattttc 480
catgtggata tggcaaagaa ggagacggtc tggcggcttg aagaatttgg acgatttgcc 540
agctttgagg ctcaaggtgc attggccaac atagctgtgg acaaagccaa cctggaaatc 600
atgacaaagc gctccaacta tactccgatc accaattaa 639

<210> 27 CO596
<211> 636
<212> DNA
<213> Homo sapiens

<400> 27
atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
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ctgaatcctg accaatcagg cgagtttatg tttgactttg atggtgatga gattttccat 480
gtggatatgg caaagaagga gacggtctgg cggcttgaag aatttggacg atttgccagc 540
tttgaggctc aaggtgcatt ggccaacata gctgtggaca aagccaacct ggaaatcatg 600
acaaagcgct ccaactatac tccgatcacc aattaa 636

<210> 28 CO595
<211> 633
<212> DNA
<213> Homo sapiens

<400> 28
atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggag gtggaggcat caaagaagaa catgtgatca tccaggccga gttctatctg 420
aatcctgacc aatcaggcga gtttatgttt gactttgatg gtgatgagat tttccatgtg 480
gatatggcaa agaaggagac ggtctggcgg cttgaagaat ttggacgatt tgccagcttt 540

gaggctcaag gtgcattggc caacatagct gtggacaaag ccaacctgga aatcatgaca 600
aagcgctcca actatactcc gatcaccaat taa 633

<210> 29 CO593
<211> 642
<212> DNA
<213> Homo sapiens

<400> 29
atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgagtct accctgaggt aactgtcatc aaagaagaac atgtgatcat ccaggccgag 420
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<211> 663
<212> DNA
<213> Homo sapiens

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35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu Gly Gly Glu Asp Asp
115 120 125

Glu Ala Asp His His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro
130 135 140

Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe
145 150 155 160

His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe
165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala
180 185 190

Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr
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Pro Ile Thr Asn
210

<210> 38 CO597
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35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Ser Gly Gly
115 120 125

Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro
130 135 140

Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe
145 150 155 160

His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe
165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala
180 185 190

Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr
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Pro Ile Thr Asn
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<210> 39 CO596
<211> 212
<212> PRT

<213> Homo sapiens

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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Gly Ser Ile
115 120 125

Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp
130 135 140

Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His
145 150 155 160

Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly
165 170 175

Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val
180 185 190

Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro
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Ile Thr Asn
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<210> 40 CO595
<211> 211
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Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Gly Ile Lys
115 120 125

Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln
130 135 140

Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val
145 150 155 160

Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg
165 170 175

Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp
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Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile
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Thr Asn
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<210> 41 CO593
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35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val Tyr Pro Glu Val Thr
115 120 125

Val Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn
130 135 140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile
145 150 155 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu
165 170 175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile
180 185 190

Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr
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Thr Pro Ile Thr Asn
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35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe Asp Ala Pro Ser Pro
115 120 125

Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu
130 135 140

Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu
145 150 155 160

Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu
165 170 175

Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn
180 185 190

Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn
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Tyr Thr Pro Ile Thr Asn
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<210> 43 CO584
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
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Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser Gly Gly Ser Gly Gly
115 120 125

Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn
130 135 140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile
145 150 155 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu
165 170 175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile
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Thr Pro Ile Thr Asn
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<400> 44
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
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Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly
115 120 125

Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn
130 135 140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile
145 150 155 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu
165 170 175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile
180 185 190

Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr
195 200 205

Thr Pro Ile Thr Asn
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<210> 45 CO582
<211> 217
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Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
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Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly
115 120 125

Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe
130 135 140

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly
145 150 155 160

Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg
165 170 175

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu
180 185 190

Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg
195 200 205

Ser Asn Tyr Thr Pro Ile Thr Asn
210 215

<210> 46 CO581
<211> 217
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg

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Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Pro Gly Gly
115 120 125

Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe
130 135 140

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly
145 150 155 160

Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg
165 170 175

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu
180 185 190

Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg
195 200 205

Ser Asn Tyr Thr Pro Ile Thr Asn
210 215

<210> 47 CO580
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
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Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile
130 135 140

Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe
145 150 155 160

Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu
165 170 175

Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala
180 185 190

Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile
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Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
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<210> 48 CO567
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<400> 48
Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu Glu His Val Ile Ile
130 135 140

Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe
145 150 155 160

Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu
165 170 175

Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala
180 185 190

Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile
195 200 205

Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
210 215 220

IMMUNE MEDIATORS AND RELATED METHODS

ABSTRACT OF THE DISCLOSURE

The present invention is directed to recombinant DNA constructs that encode soluble, fused MHC heterodimers that may or may not be further linked to an antigenic peptide.

Exhibit B

09058-005650US

LLBustian

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60/204,249	05/15/2000		150	14058-005650US	8		

20350
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Applicant(s)

Zhu Shirley, Residence Not Provided;

Continuing Data as Claimed by Applicant

Foreign Applications

If Required, Foreign Filing License Granted 08/02/2000

–

Title

Immune mediators and related methods

Preliminary Class

Data entry by : WASHINGTON, JAMES **Team :** OIPE **Date:** 08/03/2000

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PROVISIONAL APPLICATION

Attorney Docket No. 14058-005650US

"Express Mail" Label No. EL394880176US
Date of Deposit: May 15, 2000

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Transmitted herewith for filing is a provisional patent application under CFR 1.53(c) of:

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City/State/Country)
Shirley	Zhu		

Title: IMMUNE MEDIATORS AND RELATED METHODS

Enclosed are:
[X] 4 pages of the specification (including description and claims).
[X] 8 sheet(s) of [ ] formal [X] informal drawing(s).
[X] 3 Sheets of Tables.
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Respectfully submitted,

TOWNSEND and TOWNSEND and CREW LLP

_____
Kevin Bastian
Reg. No. 34,774
Attorneys for Applicant

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SF 1096746 v1

Attorney Docket No.: 14058-005650US

PROVISIONAL

PATENT APPLICATION

IMMUNE MEDIATORS AND RELATED METHODS

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IMMUNE MEDIATORS AND RELATED METHODS

CROSS-REFERENCES TO RELATED APPLICATIONS

The present application is related to U.S. Serial No. 09/261811, filed March 3, 1999, which is a continuation-in-part of U.S. Serial No. 08/480,002, filed June 7, 1995, now abandoned, U.S. Serial No 08/657,581 filed June 7, 1996, now abandoned and U.S. Serial No 09/184,692 November 2, 1998, all of which are incorporated herein by reference.

SUMMARY OF THE INVENTION

There is currently a great interest in developing pharmaceuticals based on the growing understanding of the structure and function of the major histocompatibility complex (MHC) antigens. These cell surface glycoproteins are known to play an important role in antigen presentation and in eliciting a variety of T cell responses to antigens. Identification of synthetic antigenic peptides, and demonstration that these peptides bind selectively to MHC molecules associated with disease and that stimulates T cells would help to implicate a particular peptide or peptide:MHC complex in susceptibility to an autoimmune disease. In particular, the development of soluble, fused MHC heterodimer:peptide complexes would be particularly useful in treatment of a number of diseases associated with antigen presentation by MHC molecules.

The present invention provides recombinant DNA constructs that encode soluble, fused MHC heterodimers that may or may not be further linked to an antigenic peptide. Typically, the constructs comprise a first DNA segment encoding at least a portion of a first domain of a selected MHC molecule; a second DNA segment encoding at least a portion of a second domain of the selected MHC molecule; a first linker DNA segment and connecting in-frame the first and second DNA segments; wherein linkage of the first DNA segment to the second DNA segment by the first linker DNA segment results in a fused first DNA-first linker-second DNA polysegment. The constructs of the invention may also comprise a third DNA segment encoding an antigenic peptide capable of associating with a peptide binding groove of the selected MHC molecule and a second linker DNA segment connecting in-frame the third DNA segment to the fused first DNA-first linker-second DNA polysegment.

The present invention provides soluble-fused heterdimers that comprise an additional polypeptide sequence that allows for inter-chain dimerization of the heterodimers of the invention. The sequence can be any sequence that allows for

covalent or non-covalent linkages between the molecules of the invention. As shown below, a preferred means for carrying this out is through use of segments form immunoglobulin family proteins (*e.g.*, antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds. An example shown below is the use of the constant region of the Kappa chain of an antibody. One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose.

In the present study, monomeric and dimeric forms of recombinant I-AS-peptide complexes, fused to an antigenic MBP 90-101 peptide with flexible linkers were constructed. The recombinant I-AS proteins share structural similarity to that of crystallized native MHC class II protein by protein modeling. The recombinant proteins were expressed in *E. coli* and in an insect expression system and purified by affinity chromatography and FPLC. The purified recombinant I-AS proteins showed *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant I-AS fusion proteins in the EAE model using susceptible SJL mice shows that treatment with the recombinant I-AS proteins prevents mortality and significantly reduces paralysis induced by myelin homogenate. Histological examination of sections from animal spinal cord reveals that these treatments also reduce the inflammatory lesions. In conclusion, these studies suggest that the recombinant I-AS fusion proteins have therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

Figure 1. Structure of the recombinant I-AS .MBP.β1α1 (monomer) and I-AS.MBP.β1β2α1α2.CK (dimer) proteins

Figure 2. Structural comparison of the recombinant I-AS proteins with the crystallized native murine MHC class II protein, I-EK. White color: murine MHC class II, I-EK, Yellow color: murine MHC class II, I-AS , Green color: antigenic peptide, Red color: linkers between antigenic peptide and β1 domain of I-AS or between β2 and αl domains of I-AS or between a2 and IgG Ck fragment. Blue color: IgG Ck domains. The alpha helix and beta sheets folded from the both I-AS fusion proteins are very similar to the crystallized native murine MHC class II, I-EK. The I-AS.MBP.β1β2α1α2.CK protein forms a dimer.

Figure 3. Silver staining and Western analysis of the purified recombinant I-AS monomer and dimer. Upper left, silver stained gel with and without MBP 90-101 peptide fusion of monomeric I-AS proteins. Upper-middle and upper-right,

Western analysis of monomeric I-AS proteins blotted with anti murine MHC class II protein, 10-2.16 and anti-MBP 90-101. Lower-left, silver-stained gel of purified dimeric I-AS protein. Lower-middle, Western analysis blotted with goat anti mouse IgG specific to the Ck region. Lower-right, glycosylation studies of the dimer molecules expressed in insect cells blotted with goat anti-mouse IgG specific to the Ck region.

Table 1. Summary of recombinant I-AS protein expression and purification in E. coli and Baculo-viral infected high 5 cells.

Figure 4. Diagram of the in vitro biological activity assay for the recombinant I-AS proteins.

Figure 5 In vitro biological activities of the recombinant I-AS proteins compared with APC+ antigenic peptide in the mouse T cell clone, HS-1. A. I-AS.MBP.β1α1, monomer, B. I-AS.MBP.Ck, dimer, and C. APC+ antigenic peptide. The positive (anti-CD3) and the negative (HS-1 cell alone) controls are also shown in each panel.

Figure 6. Diagram of the EAE model and standard for EAE scoring.

Figure 7. The EAE model studies. The recombinant I-AS proteins were administered to SJL mice on day 1, 4, 7, and II by i.v. injection after inducing the disease with myelin emuksified in CFA. The animals were evaluated for neurological dysfunction. Panel A: Untreated, injected with same amount of PBS solution. Panel B. Treated with the recombinant I-AS.MBP.Ck protein, a dimer form. Panel C: Treated with the recombinant I-AS.β1α1. This recombinant molecule does not carry the antigenic peptide. Panel D: Treated with the recombinant I-AS.MBP.β1α1, a monomer form.

Table 2. Summary of reduction of EAE incidences in SJL mice by administration of recombinant I-AS proteins.

Figure 8. The representative spinal cord sections of histological examination from SJL mice in the EAE model studies. Spinal cords were removed, fixed in formalin solution and embedded in paraffin. Sections were cut, stained with hemotoxylin, eosin and graded for inflammatory lesions. A. Section of spinal cord from untreated mouse; B. Section of spinal cord from mouse treated with the recombinant I-AS.MBP.Ck; C. Section of spinal cord from mouse treated with the recombinant I-AS.β1α1 without antigenic peptide fusion; D. Section of spinal cord from mouse treated with the recombinant I-AS.MBP.β1α1. The histology score for each section is marked.

Table 3. Summary of histology data from spinal cord sections.

The amino acid sequence of the I-As MBP.β1β2α1α2.Cκ shown in Figure 1 is as follows:

>

METDTLLLWVLLLWVPGSTGDFKNIVTPRTPPPASGGGGSGGGGDSERHFVFQFKGECYFTNGTQRI
RSVDRYIYNREEYLRFDSDVGEYRAVTELGRPDPEYYNKQYLEQTRAELDTVCRHNYEGVETHTSLR
RLEQPNVVISLSRTEALNHHNTLVCSVTDFYPAKIKVRWFRNGQEETVGVSSTQLIRNGDWTFQVLVM
LEMTPRRGEVYTCHVEHPSLKSPITVEWTSGGGGSGGGGSGGGGSGGGGSSSEDDIEADHVGVYG
TTVYQSPGDIGQYTHEFDGDEWFYVDLDKKETIWMLPEFGQLTSFDPQGGLQNIATGKYTLGILTKRS
NSTPATNEAPQATVFPKSPVLLGQPNTLICFVDNIFPPVINITWLRNSKSVTDGVYETSFLVNRDHSFHK
LSYLTFIPSDDDIYDCKVEHWGLEEPVLKHWASGGGGSGGGGADAAPTVSIFPPSSEQLTSGGASVV
CFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKT
STSPIVKSFNRNEC.

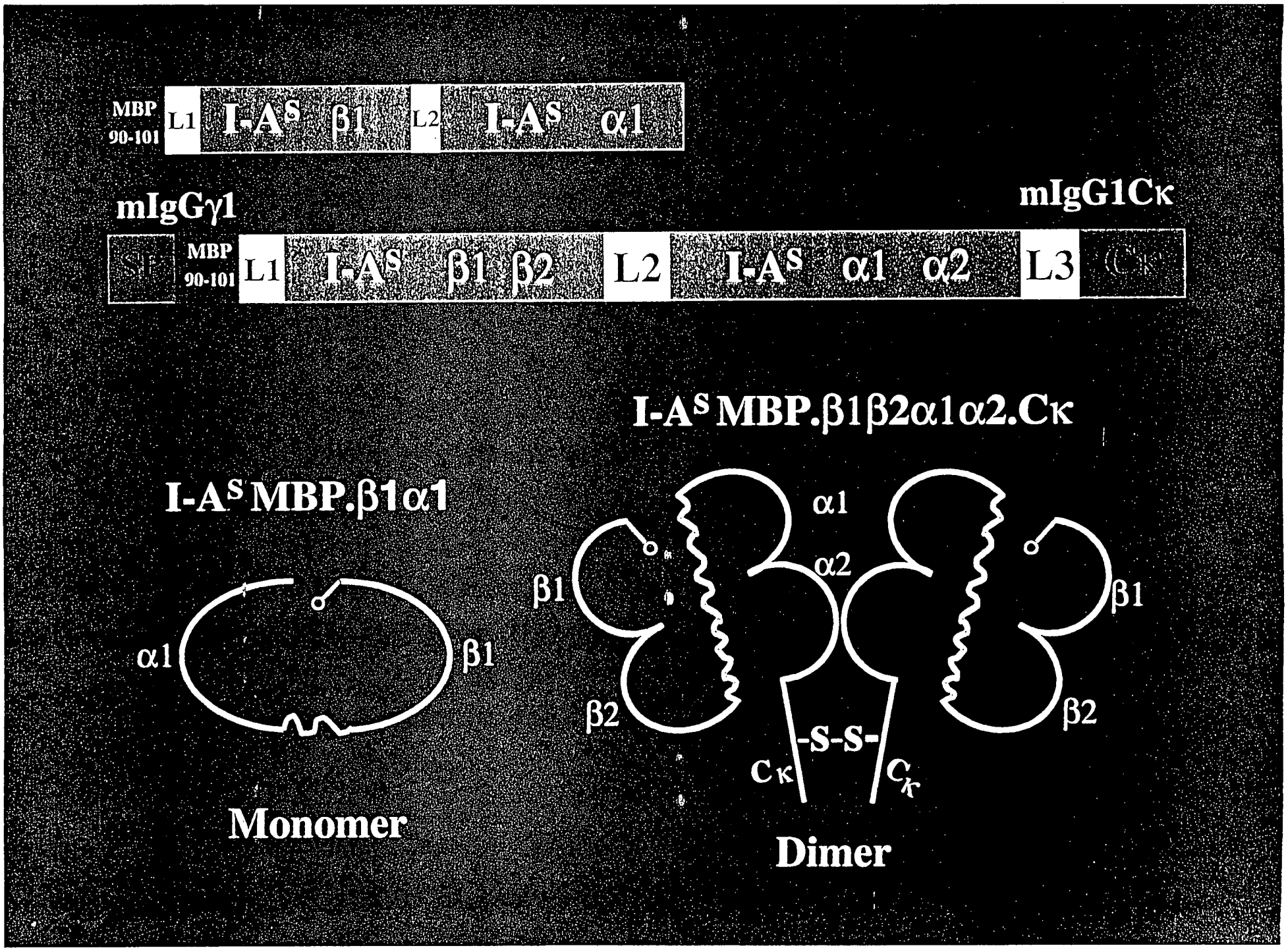
MBP 90-101
L1
I-A^S β1
L2
I-A^S α1
mIgGγ1
mIgG1Cκ
SP
MBP 90-101
L1
I-A^S β1 β2
L2
I-A^S α1 α2
L3
Cκ
I-A^{S}MBP.β1α1
I-A^{S}MBP.β1β2α1α2.Cκ
α1
β1
Monomer
α1
α2
β1
β1
β2
β2
Cκ
-S-S-
Cκ
Dimer

Figure 1

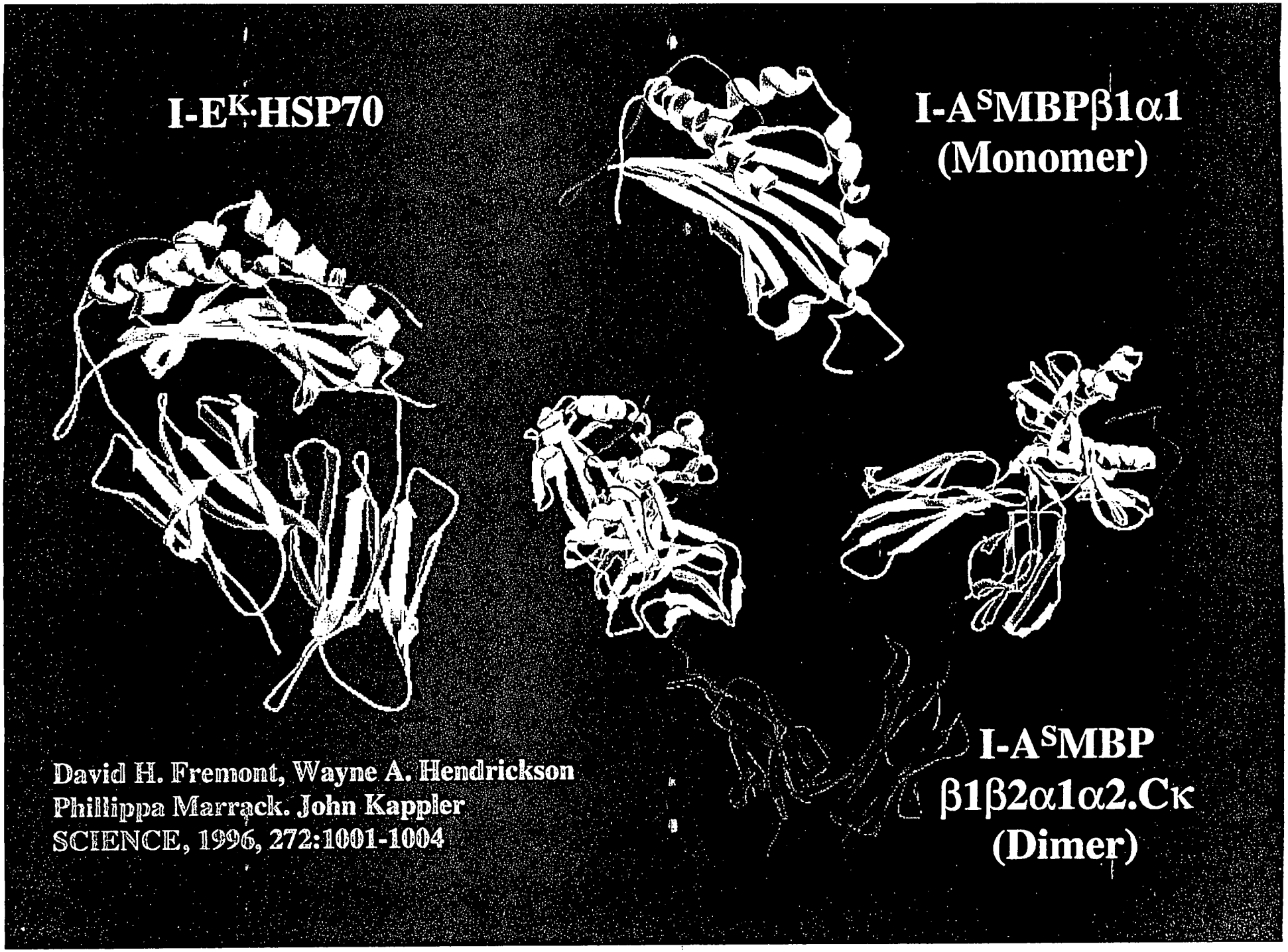
I-E^{K}-HSP70
I-A^{S}MBPβ1α1
(Monomer)
David H. Fremont, Wayne A. Hendrickson
Phillippa Marrack. John Kappler
SCIENCE, 1996, 272:1001-1004
I-A^{S}MBP
β1β2α1α2.Cκ
(Dimer)

Figure 2

Silver Staining and Western Analysis of Recombinant I-A^{S}.MBP.β1α1 Proteins

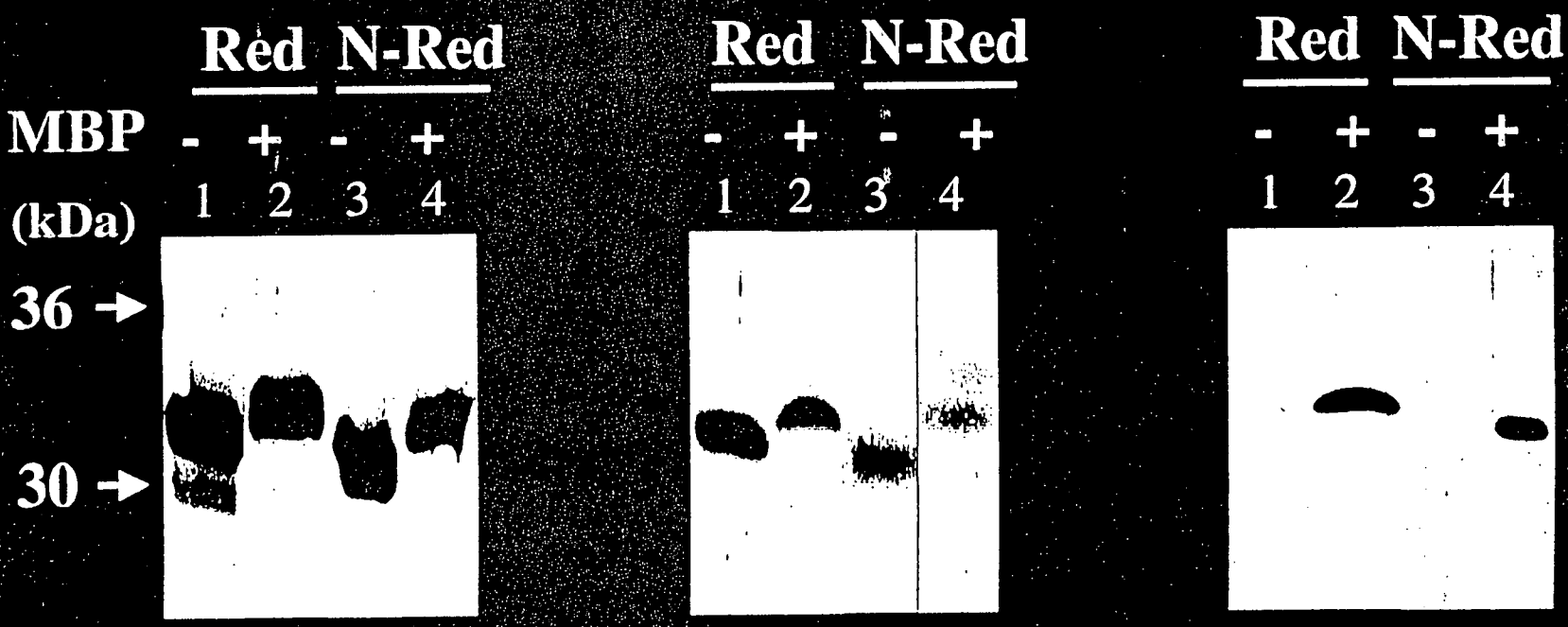

Figure 3

Table 1. Summary of Recombinant I-A^S Protein Expression and Purification

Protein	Expression System	Protein localization	Expression Level	Method for Purification
I-A^SMBP $\beta1\alpha1$	E.coli	Inclusion Body	>100 mg/L	FPLC: ion-EX & size exclusion
I-A^SMBP Ck	Baculo-High 5	Secreted form	20-25 mg/L	IgG affinity Chromatography

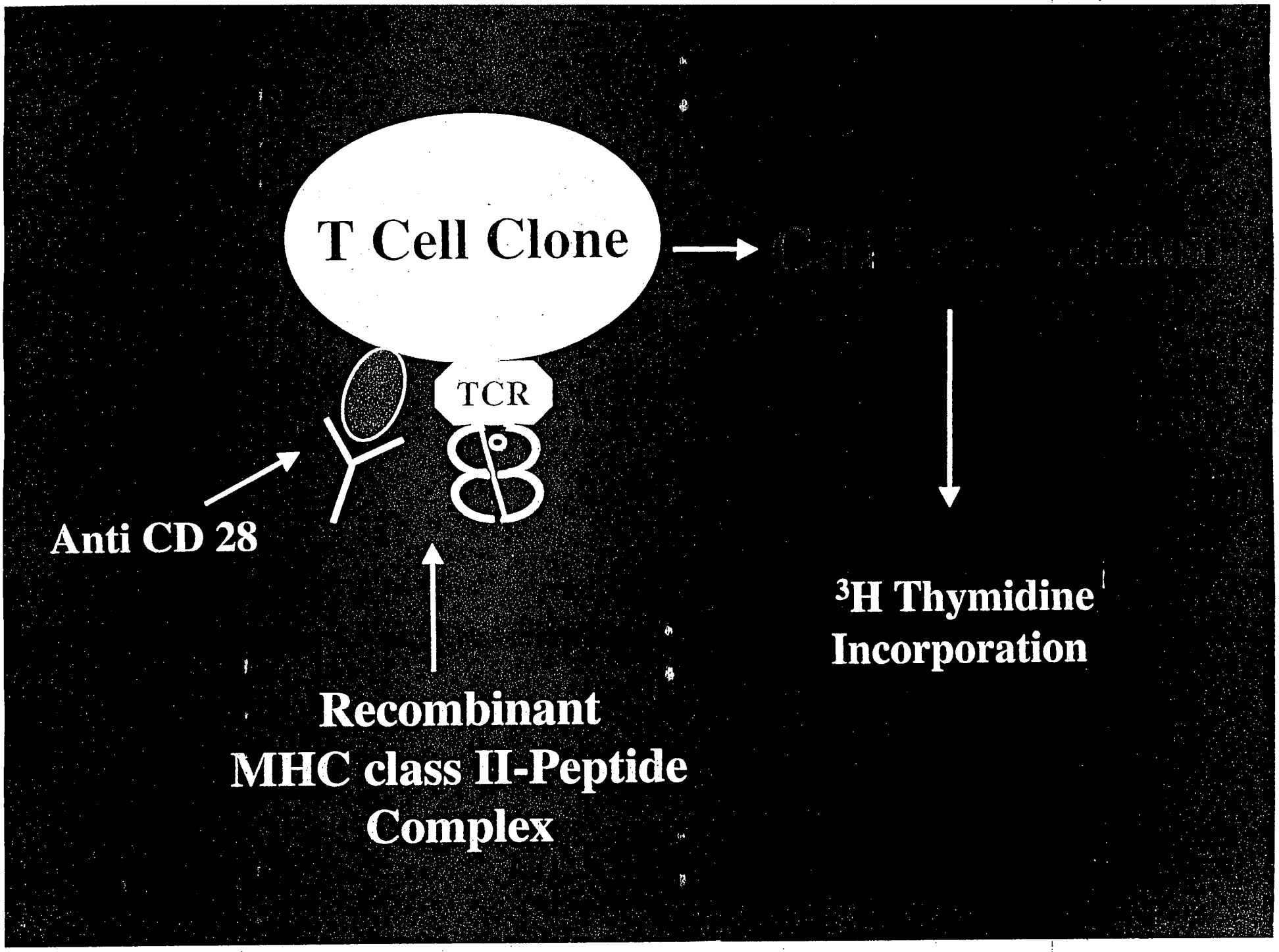
T Cell Clone
TCR
Anti CD 28
Recombinant
MHC class II-Peptide
Complex
^{3}H Thymidine
Incorporation

Figure 4

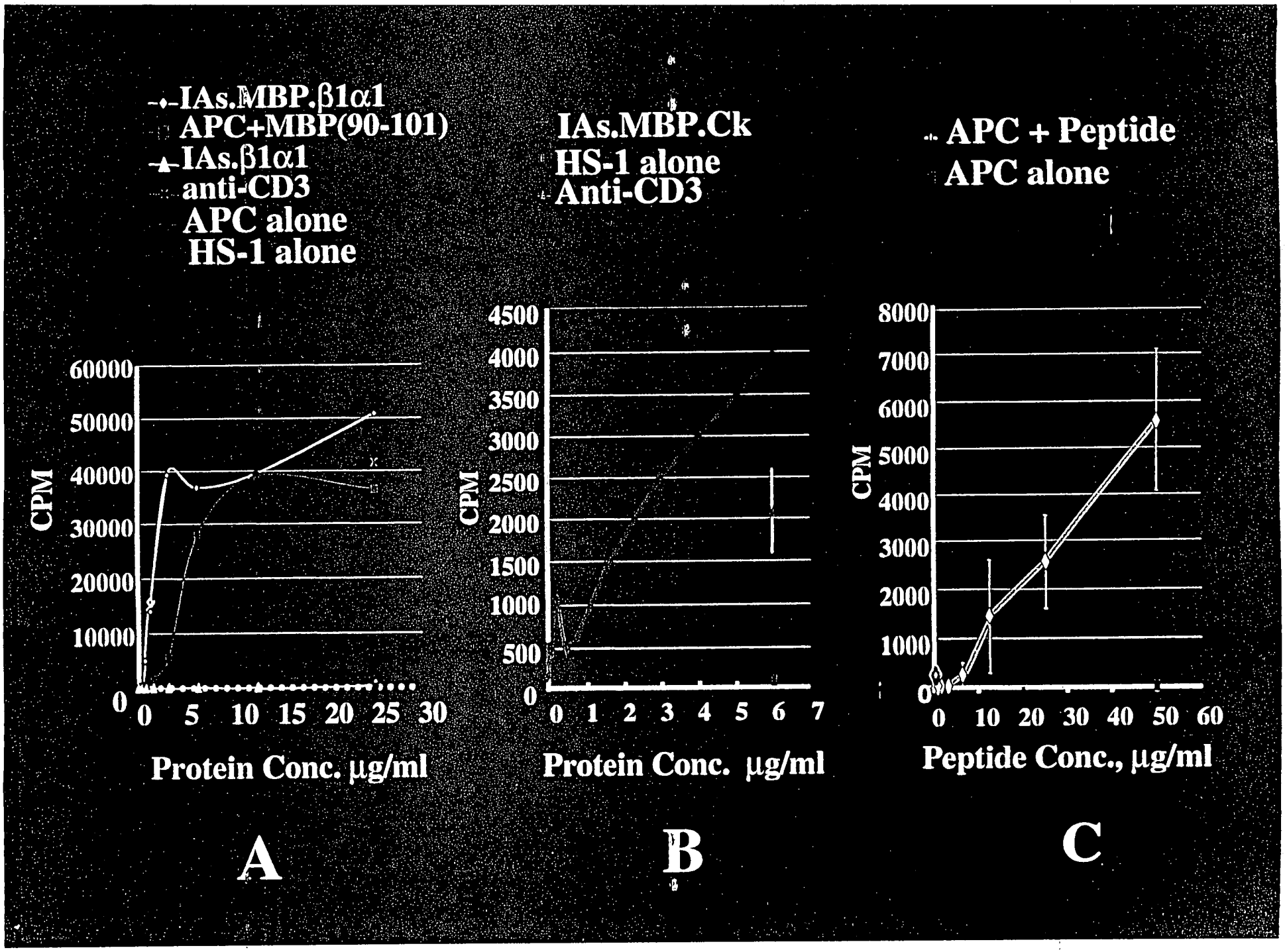
IAs.MBP.β1α1
APC+MBP(90-101)
IAs.β1α1
anti-CD3
APC alone
HS-1 alone
CPM
60000
50000
40000
30000
20000
10000
0
0 5 10 15 20 25 30
Protein Conc. µg/ml
A
IAs.MBP.Ck
HS-1 alone
Anti-CD3
CPM
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7
Protein Conc. µg/ml
B
APC + Peptide
APC alone
CPM
8000
7000
6000
5000
4000
3000
2000
1000
0
0 10 20 30 40 50 60
Peptide Conc., µg/ml
C

Figure 5

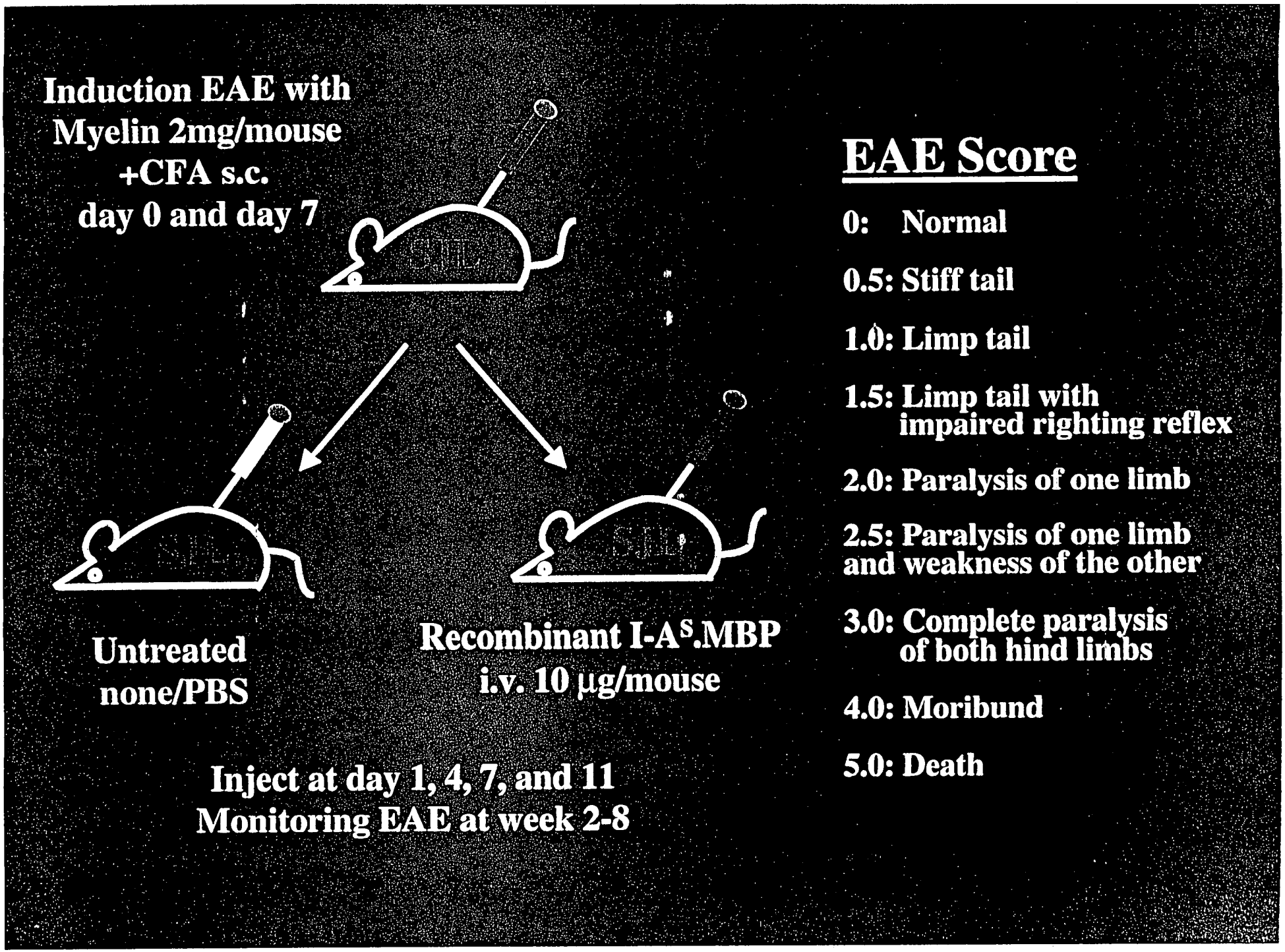
Induction EAE with
Myelin 2mg/mouse
+CFA s.c.
day 0 and day 7
Untreated
none/PBS
Recombinant I-A^{s}.MBP
i.v. 10 μg/mouse
Inject at day 1, 4, 7, and 11
Monitoring EAE at week 2-8
EAE Score
0: Normal
0.5: Stiff tail
1.0: Limp tail
1.5: Limp tail with impaired righting reflex
2.0: Paralysis of one limb
2.5: Paralysis of one limb and weakness of the other
3.0: Complete paralysis of both hind limbs
4.0: Moribund
5.0: Death

Figure 6

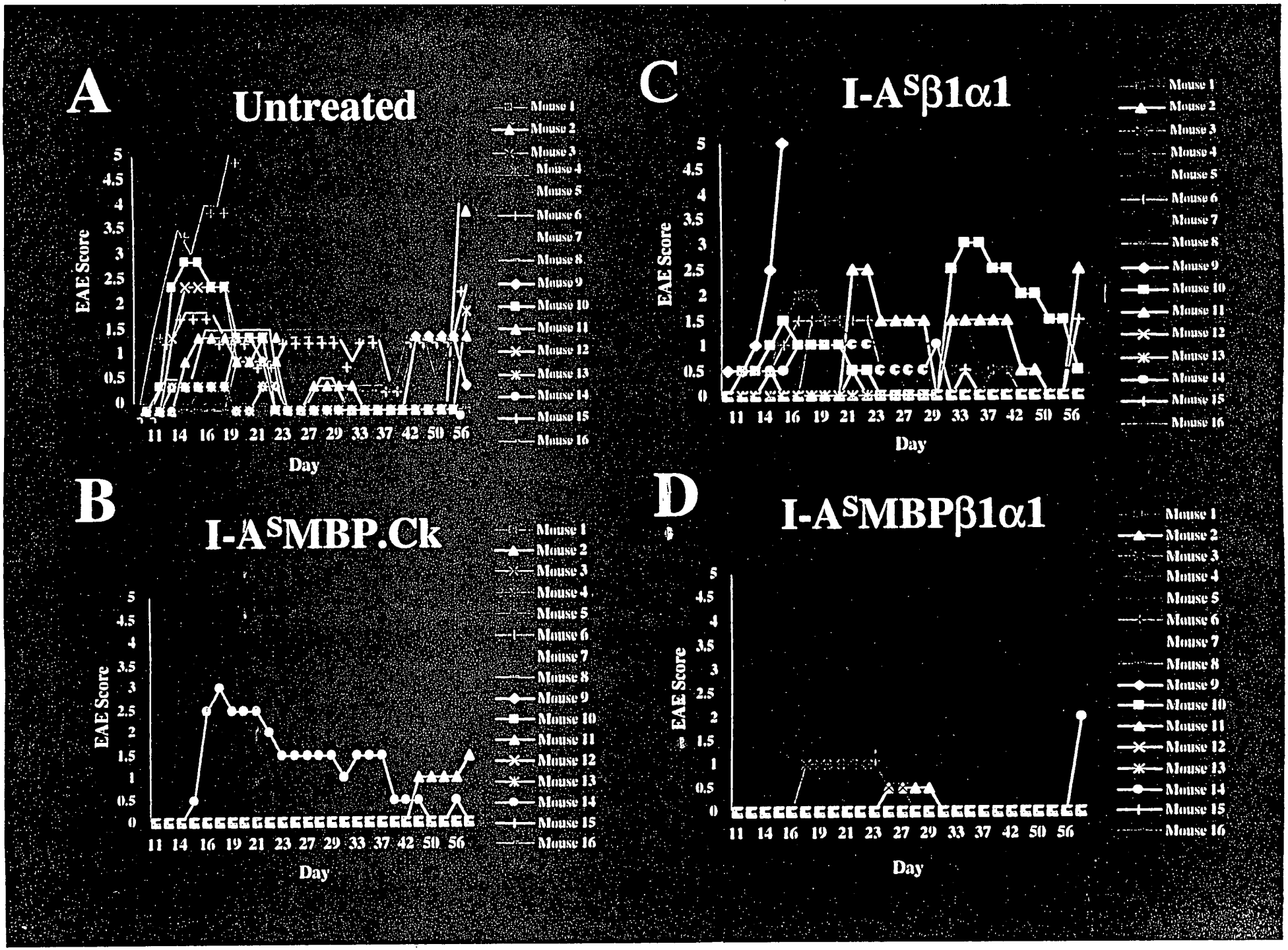
A
Untreated
B
I-A^{S}MBP.Ck
C
I-A$^{S}\beta1\alpha1$
D
I-A^{S}MBP$\beta1\alpha1$
EAE Score
Day
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
11 14 16 19 21 23 27 29 33 37 42 50 56
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
Mouse 11
Mouse 12
Mouse 13
Mouse 14
Mouse 15
Mouse 16

Figure 7

Table 2. Summary of Reduction of EAE in SJL mice by Administration of Recombinant I-A^S Proteins

Treatment	Mice with disease	
myelin/CFA+ none/ PBS	45/76	(59.2%)
myelin/CFA+ IAs.MBP.β1α1	7/56	(12.5%)
myelin/CFA + IAs.MBP.Ck	4/28	(14.3%)
myelin/CFA + IAs.β1α1	16/32	(50.0%)

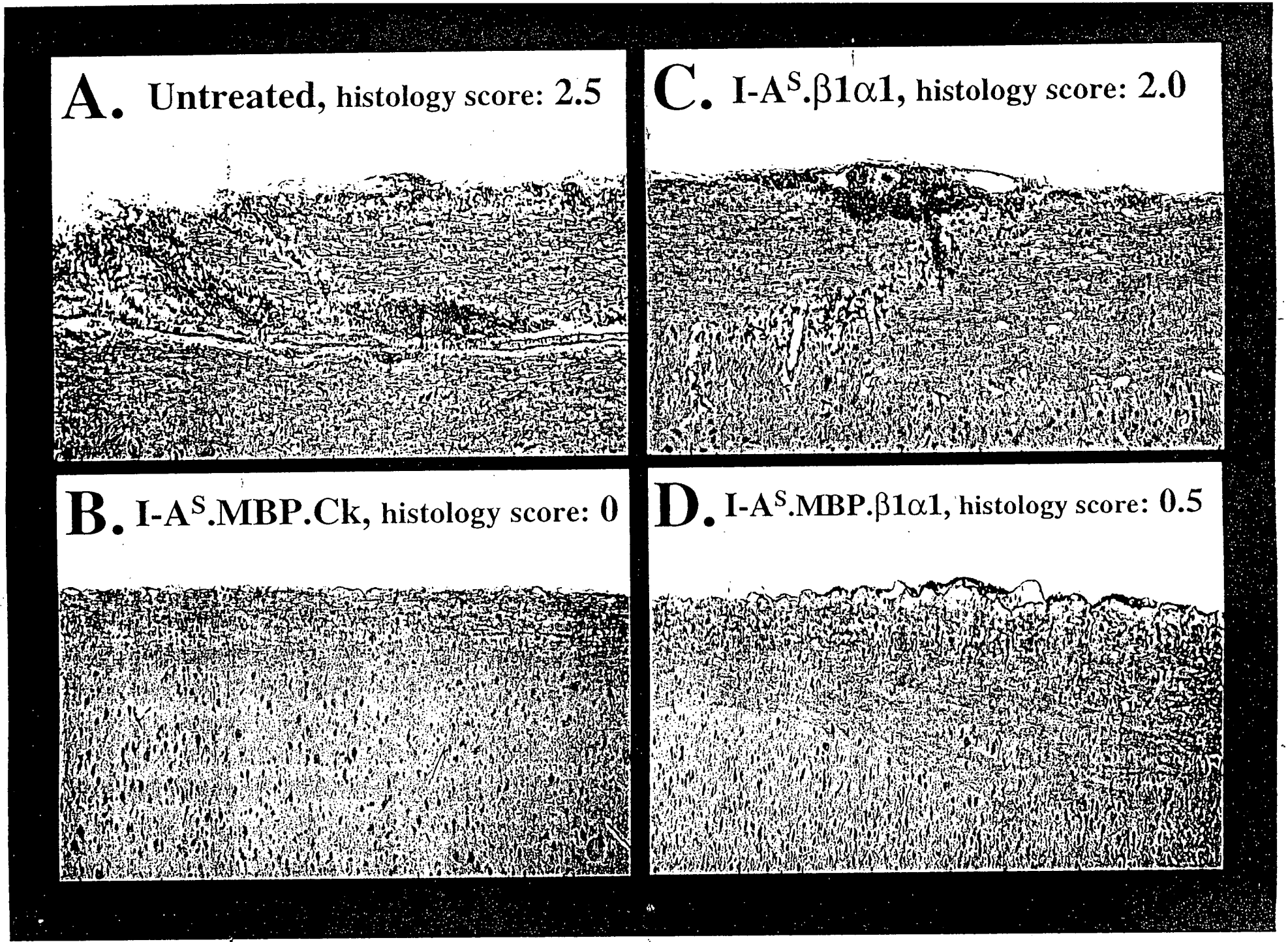
A. Untreated, histology score: 2.5
C. I-A^S.β1α1, histology score: 2.0
B. I-A^S.MBP.Ck, histology score: 0
D. I-A^S.MBP.β1α1, histology score: 0.5

Figure 8

Exhibit E

014058-005660US

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60/264,003	01/23/2001		150	014058-005660US	13		

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Applicant(s)

Darrick Carter, Seattle, WA ;
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Continuing Data as Claimed by Applicant

Foreign Applications

If Required, Foreign Filing License Granted 03/15/2001

Title

Immune mediators and related methods

Preliminary Class

Data entry by : PROCTOR, DARLENE **Team :** PCT **Date:** 03/16/2001

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Attorney Docket No.: 14058-005660US
Corixa Docket numbers: Cx-00-0020
Cx-00-0200
Cx-00-0231
Cx-00-0255
Cx-00-0359
Cx-00-0392

PROVISIONAL

PATENT APPLICATION

IMMUNE MEDIATORS AND RELATED METHODS

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Entity: Large

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Tel: 415-576-0200

IMMUNE MEDIATORS AND RELATED METHODS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is related to U.S. Serial No. 60/191,274, filed March 22, 2000; and U.S. Serial No. 60/204,249, filed May 15, 2000. Each of the aforementioned applications are herein incorporated by reference in their entirety.

This application is also related to U.S. Serial No. 09/261,811, filed March 3, 1999; which is a continuation of U.S. Serial No. 08/657,581, filed June 7, 1996, now abandoned; which is a continuation in part of U.S. Serial No. 08/480,002, filed June 7, 1995, now abandoned; U.S. Serial No 09/184,692, filed November 2, 1998, now abandoned; U.S. Serial No. 08/483,241, filed June 7, 1995; U.S. Serial No. 08/482,133, filed June 7, 1995; and U.S. Provisional Application No. 60/005,964, filed October 27, 1995.

BACKGROUND OF THE INVENTION

T cells, unlike B cells, do not directly recognize antigens. Instead, an accessory cell must first process an antigen and present it in association with an MHC molecule in order to elicit a T cell-mediated immunological response. The major function of MHC glycoproteins appears to be the binding and presentation of processed antigen in the form of short antigenic peptides.

In addition to binding foreign or "non-self antigenic peptides," MHC molecules can also bind "self" peptides. If T lymphocytes then respond to cells presenting "self" or autoantigenic peptides, a condition of autoimmunity results. Over 30 autoimmune diseases are presently known, including myasthenia gravis (MG), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), insulin-dependent diabetes mellitus (IDDM) , etc. Characteristic of these diseases is an attack by the immune system on the tissues of the host. In non-diseased individuals, such attack does not occur because the immune system recognizes these tissues as "self." Autoimmunity occurs when a specific adaptive immune response is mounted against self tissue antigens.

There is therefore currently a great interest in developing pharmaceuticals based on the growing understanding of the structure and function of the major histocompatibility complex (MHC) antigens. Identification of synthetic autoantigenic

peptides, and demonstration that these peptides bind selectively to MHC molecules associated with disease and that stimulates T cells would help to implicate a particular peptide or peptide:MHC complex in susceptibility to an autoimmune disease. In particular, the development of soluble, fused MHC heterodimer:peptide complexes would be particularly useful in treatment of a number of diseases associated with antigen presentation by MHC molecules. Furthermore, the development of single chain, multimeric complexes would be of interest (*see, e.g.*, WO 93/10220, WO 98/05684, WO 97/35991, WO 98/03552, WO 99/13095, WO 98/06749, WO 99/09064, and U.S. Patent 5,869,270).

SUMMARY OF THE INVENTION

The present invention provides recombinant nucleic acid constructs that encode single chain, recombinant MHC class II heterodimers (e.g., "soluble, fused heterodimers") that may or may not be further linked to an antigenic peptide. The constructs of the invention are dimerized or multimerized by inter-chain dimerization or multimerization of the single chain heterodimers of the invention. The dimerization or multimerization sequence can be any sequence that allows for covalent or non-covalent linkages between the molecules of the invention, e.g., by direct chemical means or by recombinant means. As shown below, a preferred means for carrying this out is through use of segments from immunoglobulin family proteins (e.g., antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds. In another embodiment, a leucine zipper domain forms a non-covalent linkage. An example shown below is the use of the constant region of the kappa chain of an antibody. One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose. The single chain molecules of the invention thus can be multimers wherein each single chain heterodimer is from a different MHC class II allele. In addition, each heterodimer in the multimer can be bound to a different antigen.

In one embodiment, monomeric and dimeric forms of recombinant mouse I-AS-peptide complexes, fused to an antigenic MBP 90-101 peptide with flexible linkers were constructed. The recombinant I-AS proteins share structural similarity to that of crystallized native human MHC class II protein as determined by protein modeling. The recombinant proteins were expressed in *E. coli* and in an insect expression system and

purified by affinity chromatography and FPLC. The purified recombinant I-AS proteins showed *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant I-AS fusion proteins in the EAE model using susceptible SJL mice shows that treatment with the recombinant I-AS proteins prevents mortality and significantly reduces paralysis induced by myelin homogenate. Histological examination of sections from animal spinal cord reveals that these treatments also reduce the inflammatory lesions. These results demonstrate that the single chain MHC class II heterodimers have therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

In another embodiment, novel linkers are provided for forming single chain MHC class II heterodimers. These linkers can be used with the multimer constructs described above. In another embodiment, the constructs of the invention are optimized for prokaryotic expression, using codons adjusted for *E. coli* codon bias.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic structure of the recombinant I-AS .MBP.β1α1 (monomer) and I-AS.MBP.β1β2α1α2.CK (dimer) proteins.

Figure 2. Structural comparison of the recombinant I-AS proteins with the crystallized native murine MHC class II protein, I-EK. White color: murine MHC class II, I-EK, Yellow color: murine MHC class II, I-AS , Green color: antigenic peptide, Red color: linkers between antigenic peptide and β1 domain of I-AS or between β2 and αl domains of I-AS or between a2 and IgG Ck fragment. Blue color: IgG Ck domains. The tertiary structure predicted for both I-AS fusion proteins are very similar to the crystallized native murine MHC class II, I-EK. The I-AS.MBP.β1β2α1α2.CK protein can form a dimer.

Figure 3. Silver staining and western analysis of the purified recombinant I-AS monomer and dimer. Left, silver stained gel with and without MBP 90-101 peptide fusion of monomeric I-AS proteins. Middle, western analysis of monomeric I-AS proteins blotted with anti murine MHC class II protein, 10-2.16. Right, western analysis of monomeric I-AS proteins blotted anti-MBP 90-101.

Figure 4. Diagram of the *in vitro* biological activity assay for the recombinant I-AS proteins.

Figure 5. *In vitro* biological activities of the recombinant I-AS proteins compared with APC+ antigenic peptide in the mouse T cell clone, HS-1. A. I-AS.MBP.β1α1, monomer, B. I-AS.MBP.Ck, dimer, and C. APC+ antigenic peptide. The positive (anti-CD3) and the negative (HS-1 cell alone) controls are also shown in each panel.

Figure 6. Diagram of the EAE model and standard for EAE scoring.

Figure 7. The EAE model studies. The recombinant I-AS proteins were administered to SJL mice on day 1, 4, 7, and II by i.v. injection after inducing the disease with myelin emulsified in CFA. The animals were evaluated for neurological dysfunction. Panel A: Untreated, injected with same amount of PBS solution. Panel B. Treated with the recombinant I-AS.MBP.Ck protein, a dimer form. Panel C: Treated with the recombinant I-AS.β1α1. This recombinant molecule does not carry the antigenic peptide. Panel D: Treated with the recombinant I-AS.MBP.β1α1, a monomer form.

Figure 8. The representative spinal cord sections of histological examination from SJL mice in the EAE model studies. Spinal cords were removed, fixed in formalin solution and embedded in paraffin. Sections were cut, stained with hematoxylin, eosin and graded for inflammatory lesions. A. Section of spinal cord from untreated mouse; B. Section of spinal cord from mouse treated with the recombinant I-AS.MBP.Ck; C. Section of spinal cord from mouse treated with the recombinant I-AS.β1α1 without antigenic peptide fusion; D. Section of spinal cord from mouse treated with the recombinant I-AS.MBP.β1α1. The histology score for each section is marked.

Figure 9 shows a schematic representation of a truncated single chain MHC class II peptide complex that is a dimer with two peptide specificities.

Figure 10 shows a schematic representation of a single chain MHC class II peptide complex that is a dimer with two peptide specificities.

Figure 11 shows a schematic representation of an single chain MHC class II peptide complex that is a tetramer with four peptide specificities.

Figure 12 shows a schematic representation of an single chain MHC class II peptide complex that is a tetramer with two peptide specificities and two different MHC class II alleles.

Figure 13 shows the effect of different recombinant MHC class II molecules on the development of EAD (day 60+).

DETAILED DESCRIPTION OF THE INVENTION

Introduction

The present invention provides recombinant DNA constructs that encode soluble, fused MHC class II heterodimers (i.e., single chain MHC class II heterodimers) that may or may not be further linked to an antigenic peptide. Typically, the constructs comprise a first DNA segment encoding a β1 domain of a selected MHC class II molecule; a second DNA segment encoding a α1 domain of the selected MHC class II molecule; and a first linker DNA segment connecting in-frame the first and second DNA segments; wherein linkage of the first DNA segment to the second DNA segment by the first linker DNA segment results in a fused first DNA-first linker-second DNA polysegment. The constructs of the invention may also comprise a third DNA segment encoding an antigenic peptide capable of associating with a peptide binding groove of the selected MHC class II molecule and a second linker DNA segment connecting in-frame the third DNA segment to the fused first DNA-first linker-second DNA polysegment.

The present invention provides soluble-fused heterodimers that comprise an additional polypeptide sequence that allows for inter-chain dimerization of the heterodimers of the invention. The additional polypeptide allows multimerization of the single chain MHC class II molecules, to produce, e.g., dimers and tetramers. The sequence can be any sequence that allows for covalent or non-covalent linkages between the molecules of the invention. In one embodiment, the single chain molecules are covalently linked using chemical methods known to those of skill in the art, e.g., photoaffinity methods or homo-bifunctional protein cross-linkers (*see, e.g.*, Hermanson *et al., Bioconjugate Techniques*, (1996)). In one embodiment, the molecules are covalently linked using heterobifunctional protein cross-linkers. As shown below, one means for carrying this out is through use of segments form immunoglobulin family proteins (*e.g.*, antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds. An example shown below is the use of the constant region of the kappa chain of an antibody (Cκ), from either a heavy or a light chain. Other dimerization sequences include a leucine zipper, a STAT protein N-terminal domain, or the FK506 binding protein (*see, e.g.*, O'Shea, *Science* 254: 539 (1991), Barahmand-Pour *et al.*, *Curr. Top. Microbiol. Immunol.* 211:121-128 (1996); Klemm *et*

al., Annu. Rev. Immunol. 16:569-592 (1998); Ho *et al., Nature* 382:822-826 (1996)). One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose.

In addition, the multimeric, single chain class II molecules of the invention comprise at least two different MHC class II alleles that are associated with an autoimmune disease state, and/or at least two different autoantigenic peptides that are associated with a particular autoimmune disease state. In one example, the multimeric, single chain class II molecules have chains from different DR2 alleles, e.g., DRB5*0101 and DRB1*1501. In another embodiment, the autoantigenic peptides are peptides associated with multiple sclerosis, e.g., MBP (e.g., amino acid residues 83-102Y83), PLP (e.g., amino acid residues 40-60, 89-106, 95-117, and 185-206); and MOG. In addition, other antigens associated with autoimmune disease, such as acetylcholine receptor and type II collagen, can be linked to the single chain heterodimers of the invention..

In a further embodiment, the single chain class II molecules of the invention have novel linkers, as described herein. The mammalian MHC class II single chain constructs of the invention may also be constructed to use preferred prokaryotic codons, for expression, e.g., in *E. coli,* using codon preference tables and methods known to those of skill in the art.

Definitions

Prior to setting forth the invention, it may be helpful to an understanding thereof to provide definitions of certain terms to be used hereinafter:

Single chain MHC class II heterodimer: As used herein this term refers to a fusion protein such as the fused or single chain MHC class II heterodimer complex of the invention, which optionally also is fused to a peptide to form a single chain MHC class II heterodimer:peptide complex. Such fusion proteins will be indicated with a colon (:). MHC-peptide complexes which are not fusion proteins, are native MHC containing protein or exogenously loaded MHC molecules are indicated with a dash (-). The fusion proteins of the invention can also be multimers, having two, four or more single chain molecules linked covalently or non-covalently through multimerization domains in the single chain molecule. A single chain molecule of the invention typically comprises at

least an MHC class II β1 domain and an MHC class II α1 domain, optionally β1 β2 α1 α2 domains or any combination thereof in any order.

A domain of a selected MHC molecule: A portion of an MHC domain which is sufficient to form, either alone, or in combination with another portion of an MHC domain, a peptide binding site which is capable of presenting an antigenic peptide in such a fashion that it is recognized by a T cell receptor. Such MHC domains would include the extracellular portion of the two polypeptide chains of Class II MHC. This would include the α chain (α1 and α2 domains) and β chain (β1 and β2 domains) of Class II MHC. This would include β1 and α1, β1, β2 and α1, α2, α1 or α2 independent of the other, or α1 and α2 in tandem (α1α2). It would also include β1 or β2 independent of the other, or β1 and β2 in tandem (β1β2). This would also include any suitable combination of the α1, α2, β1, and β2 domains. The domains can be directly linked, or can be linked via an amino acid linker.

Linker DNA segment: A segment of DNA encoding from about 1 to about 50, preferably from about 5 to about 25 amino acids, which forms a flexible link between two DNA segments. This flexible link allows the two DNA segments to attain a proper configuration, such as an MHC peptide binding groove, or allows a peptide to properly bind into such a groove.

Antigenic peptide: The immunological properties of MHC histocompatibility proteins are largely defined by the antigenic peptide that is bound to them. An antigenic peptide is one which contains an epitope (an amino acid sequence) recognized by immune cells, e.g., T cells, and is capable of stimulating an MHC-mediated immune response.. Antigenic peptides for a number of autoimmune diseases are known. For example, in experimentally induced autoimmune diseases, antigens involved in pathogenesis have been characterized: in arthritis in rat and mouse, native type II collagen is identified in collagen-induced arthritis, and mycobacterial heat shock protein in adjuvant arthritis (Stuart *et al.*, *Ann. Rev. Immunol.* 2:199-218, 1984; and van Eden *et al.*, *Nature* 331:171-173, 1988); thyroglobulin has been identified in experimental allergic thyroiditis (EAT) in mice (Marion *et al.*, *J. Exp. Med.* 152:1115-1120, 1988); acetyl-choline receptor (AChR) in experimental allergic myasthenia gravis (EAMG) (Lindstrom *et al.*, *Adv. Immunol.* 42:233-284, 1988) ; and myelin basic protein (MBP) and proteolipid protein (PLP) in experimental allergic encephalomyelitis (EAE) in mouse and rat (Acha-

Orbea *et al.*, *Ann. Rev. Imm.* 7:377-405, 1989). In addition, target antigens have been identified in humans: type II collagen in human rheumatoid arthritis (Holoshitz *et al.*, *Lancet* ii:305-309, 1986), acetylcholine receptor in myasthenia gravis (Lindstrom *et al.*, *Adv. Immunol.* 42.:233-284, 1988), and MBP, PLP, and MOG in multiple sclerosis in humans.

MHC: The major histocompatibility complex (MHC) is a family of highly polymorphic proteins, divided into two classes, Class I and Class II, which are membrane-associated and present antigen to T lymphocytes (T cells). MHC Class I and Class II molecules are distinguished by the types of cells on which they are expressed, and by the subsets of T cells which recognize them. Class I MHC molecules (e.g., HLA-A, -B and -C molecules in the human system) are expressed on almost all nucleated cells and are recognized by cytotoxic T lymphocytes (CTL), which then destroy the antigen-bearing cells. Class II MHC molecules (HLA-DP, -DQ and -DR, for example, in humans) are expressed primarily on the surface of antigen-presenting cells, such as B lymphocytes, dendritic cells, macrophages, and the like. Class II MHC is recognized by $CD4^+$ T helper lymphocytes (T_H). T_H cells induce proliferation of both B and T lymphocytes, thus amplifying the immune response to the particular antigenic peptide that is displayed (Takahashi, *Microbiol. Immunol.*, 37:1-9, 1993).

Two distinct antigen processing pathways are associated with the two MHC classes. Intracellular antigens, synthesized inside of the cell, such as from viral or newly synthesized cellular proteins, for example, are processed and presented by Class I MHC. Exogenous antigens, taken up by the antigen-presenting cell (APC) from outside of the cell through endocytosis, are processed and presented by Class II MHC. After the antigenic material is proteolytically processed by the MHC-bearing cell, the resulting antigenic peptide forms a complex with the antigen binding groove of the MHC molecule through various noncovalent associations. The MHC-peptide complex on the cell surface is recognized by a specific T cell receptor on a cytotoxic or helper T cell.

The MHC of humans (also referred to as human leukocyte antigens (HLA)) on chromosome 6 has three loci, HLA-A, HLA-B and HLA-C, the first two of which have a large number of alleles encoding alloantigens. An adjacent region, known as HLA-D, is subdivided into HLA-DR, HLA-DQ and HLA-DP. The HLA region is now known as the human MHC region, and is equivalent to the H-2 region in mice. HLA-A, -

B and -C resemble mouse H-2K, -D, and -L and are the Class I MHC molecules. HLA-DP, -DQ and -DR resemble mouse I-A and I-E and are the Class II molecules. MHC glycoproteins of both classes have been isolated and characterized (*see Fundamental Immunology*, 2d Ed., W.E. Paul (ed.), Ravens Press, N.Y. (1989); and Roitt *et al.*, *Immunology*, 2d Ed., Gower Medical Publishing, London (1989) , which are both incorporated herein by reference).

Human MHC Class I molecules consist of a polymorphic type I integral membrane glycoprotein heavy chain of about 46 kD, noncovalently associated with a 12 kD soluble subunit, β2-microglobulin. The heavy chain consists of two distinct extracellular regions, the membrane distal, peptide binding region formed by the α1 and α2 domains, and the membrane proximal, CD8-binding region derived from the α3 domain. β_2- microglobulin is a single, compact immunoglobulin-like domain that lacks a membrane anchor, and exists either associated with the class I heavy chain or free in plasma (Germain and Margulies, *Annu. Rev. Immunol.* 11:403-50, 1993).

Human MHC Class II is a heterodimeric integral membrane protein. Each dimer consists of one α and one β chain in noncovalent association. The two chains are similar to each other, with the α chain having a molecular weight of 32-34 kD and the β chain having a molecular weight of 29-32 kD. Both polypeptide chains contain N-linked oligosaccharide groups and have extracellular amino termini and intracellular carboxy termini.

The extracellular portions of the α and β chain that comprise the class II molecule have been subdivided into two domains of about 90 amino acids each, called α1, α2, and β1, β2, respectively. The α2 and β2 domains each contain a disulfide-linked loop. The peptide-binding region of the class II molecule is formed by the interaction of the α1 and β1 domains. This interaction results in an open-ended, antigenic peptide-binding groove made up of two α helices, and an eight-stranded β-pleated sheet platform.

The α and β chains of Class II molecules are encoded by different MHC genes and are polymorphic (*see* Addas *et al.*, *Cellular and Molecular Immunology*, 2d Ed., W.B. Saunders Co., New York (1994), which is incorporated by reference in its entirety). Within the present invention, a preferred α chain is DRA*0101 and a preferred β chain is DRβ1*1501.

MHC Class II alleles

The soluble, fused heterodimer MHC class II:peptide complexes of the present invention can incorporate cDNA from any allele that predisposes or increases the likelihood of susceptibility to a specific autoimmune disease. Specific autoimmune diseases are correlated with specific MHC types. Specific haplotypes have been associated with many of the autoimmune diseases. For example, HLA-DR2$^+$ and HLA-DR3$^+$ individuals are at a higher risk than the general population to develop systemic lupus erythematosus (SLE) (Reinertsen *et al.*, *N. Engl. J. Med.* 299:515-18, 1970). Myasthenia gravis has been linked to HLA-D (Safwenberg *et al.*, *Tissue Antigens* 12:136-42,1978. Susceptibility to rheumatoid arthritis is associated with HLA-D/DR in humans. Methods for identifying which alleles, and subsequently which MHC-encoded polypeptides, are associated with an autoimmune disease are known in the art. Exemplary alleles for IDDM include DR4, DQ8, DR3, DQ3.2.

Uses of soluble, fused MHC heterodimers

Soluble, fused MHC class II heterodimer:peptide complexes of the present invention can be used as antagonists to therapeutically block the binding of particular T cells and antigen-presenting cells. In addition, the molecules can induce anergy, or proliferative nonresponsiveness, and possibly apoptosis, in targeted T cells, both *in vivo* and *in vitro*. A soluble, fused MHC heterodimer:peptide molecule directed toward a desired autoimmune disease contains the antigenic peptide implicated for that autoimmune disease properly positioned in the binding groove of the MHC molecule, without need for solubilization of MHC or exogenous loading of an independently manufactured peptide.

Previous methods for producing desirable MHC Class II histocompatibility proteins have provided material that contains a mixture of antigenic peptides (Buus *et al.*, *Science* 242:1045-1047, 1988; and Rudensky *et al.*, *Nature* 353:622-627, 1991), which can be only partially loaded with a defined antigenic peptide (Watts and McConnel, *Pro. Natl. Acad. Sci. USA* 83:9660-64, 1986; and Ceppellini *et al.*, *Nature* 339:392-94, 1989). Various methods have been developed to produce heterodimers that do not present endogenous antigens (Stern and Wiley, *Cell* 68:465-77, 1992; Ljunggren *et al.*, *Nature* 346:476-80, 1990; and Schumacher *et al.*, *Cell* 62:563-67, 1990) that can be loaded with a

peptide of choice. WO 95/23814 and Kozono *et al.* have described production of soluble murine Class II molecules, I-E^{dk} and I-A^{d}, each with a peptide attached by a linker to the N terminus of the β chain. Ignatowicz *et al.* (*J. Immunol.* 154:38-62, 1995) have expressed membrane-bound I-A^{d} with peptide attached. These methods incorporate the use of both membrane-bound heterodimer and soluble heterodimer.

The current invention offers the advantage of a soluble, fused MHC heterodimer made up of two or more MHC domains joined together via a flexible linkage, and onto which is tethered (via an additional flexible linkage) an antigenic peptide which is able to bind to the peptide binding groove presented by the soluble, fused MHC heterodimer. Such a complex provides an MHC molecule which is soluble and, because the components of the heterodimer and corresponding antigenic peptide are permanently linked into a single chain configuration, there is no need for complex heterodimer truncation or formation. These complexes eliminate inefficient and nonspecific peptide loading. Producing the claimed MHC:peptide complexes by recombinant methodology results in specific, high yield protein production, where the final product contains only the properly configured MHC:peptide complex of choice.

As used herein, a soluble heterodimer is one that does not contain membrane-associated MHC. The soluble MHC heterodimer of the present invention has never been membrane-associated. Further, the polypeptides contained within the MHC heterodimer do not contain an amino acid sequence that acts as a transmembrane domain or as a cytoplasmic domain.

The present invention therefore provides a soluble, fused MHC heterodimer which contains an antigenic peptide covalently attached to the amino terminal portion of an α or β chain of MHC through a peptide linkage, and the C terminal of the linked α or β chain may be attached to the N terminal portion of another α or β chain, there by creating a two, three, or four domain MHC molecule. The invention further provides a multimerization domain to provide a multimeric MHC class II heterodimer. The invention further provides novel linkers, and multimeric MHC class II heterodimers that are bound to different antigenic peptides.

The amino acid sequence of each of a number of Class I and Class II proteins are known, and the genes or cDNAs have been cloned. Thus, these nucleic acids can be used to express MHC polypeptides. If a desired MHC gene or cDNA is not

available, cloning methods known to those skilled in the art may be used to isolate the genes. One such method that can be used is to purify the desired MHC polypeptide, obtain a partial amino acid sequence, synthesize a nucleotide probe based on the amino acid sequence, and use the probe to identify clones that harbor the desired gene from a cDNA or genomic library.

Linkers

Linkers of the current invention may be from about 1 to about 50 amino acids in length, depending on the molecular model of the MHC or MHC:peptide complex. In one embodiment, flexible linkers are made of repeating Gly residues separated by one or more Ser residues to permit a random, flexible motion. In the case of Class II MHC complexes this flexibility accommodates positioning of the α and β segments to properly configure the binding groove, and also allows for maximum positioning of the peptide in the groove. In another embodiment, the linker comprises a CD4 binding site, as described below in the Example section (see also Table 1). In another embodiment, longer linkers between the chains contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). To inhibit the continuation of secondary structure across the linker, prolines can be added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. In another embodiment, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. Finally, a combination of these types of linkers could also be used.

Linker position and length can be modeled based on the crystal structure of MHC Class II molecules (Brown *et al., Nature* 364:33-39, 1993), where α1 and β1 are assembled to form the peptide binding groove. Linkers joining segments of the α and β chains together are based on the geometry of the region in the hypothetical binding site and the distance between the C terminus and the N terminus of the relevant segments. Molecular modeling based on the X-ray crystal structure of Class II MHC (Stern *et al., Nature* 368:215-221, 1994) dictates the length of linkers joining antigenic peptide, α chain segments and β chain segments. The recombinant portions of the molecules of the invention can also be directly linked, without additional amino acids linkers.

Identification of autoantigens

The invention also provides methods for preparing responder T-cell clones that proliferate when combined with a selected antigenic peptide presented by a stimulator cell. Such clones can be used to identify and map antigenic peptides associated with autoimmune disease. These peptides can then be incorporated into the soluble, fused MHC heterodimer:peptide complexes of the invention. The method provides isolation and enrichment of non-adherent, $CD56^-$, $CD8^-$ T cells that are reactive with a selected antigenic peptide. These cells are herein referred to as responder cells. Suitable responder cells can be isolated, for example, from peripheral blood mononuclear cells (PBMNC) obtained from patients prior to or after onset of an autoimmune disease of interest. For example, PBMNCs can be obtained from prediabetic and new onset diabetic patients. These patients can be pre-screened for specific HLA markers, such as DR3-DR4 or DQ3.2, which have the highest association with susceptibility to IDDM. From the collected PBMNCs, a portion is kept to serve as stimulator cells. From the remainder, the desired autoreactive responder cells are purified and isolated by two rounds of plating, to remove adherent cells from the population, followed by removal of monocytes and B cells with nylon wool. Enrichment for non-adherent $CD4^+$ T cells is completed by sequential plating of the cells onto plates coated with anti-CD8 and anti-CDS6 antibodies.

The stimulator cells are pulsed or primed with whole GAD or an appropriate antigenic peptide. For example, stimulator cells from the PBMNCs of IDDM patients can be stimulated with antigenic GAD peptides then combined with PBMNCs or responder cells. After seven or 14 days, responder cell (T cell) clones are generated through limiting dilution and tested for antigen reactivity.

These responder cell (T cell) clones can then be used, for example, to map epitopes which bind to MHC and are recognized by a particular T cell. One such method uses overlapping peptide fragments of the autoantigen which are generated by tryptic digestion, or more preferably, overlapping peptides are synthesized using known peptide synthesis techniques. The peptide fragments are then tested for their ability to stimulate the responder T cell clones or lines (for example, Ota *et al.*, *Nature* 346:183-187, 1990).

Once such a peptide fragment has been identified, synthetic antigenic peptides can be specifically designed, for example, to enhance the binding affinity for

MHC and to out-compete any naturally processed peptides. Such synthetic peptides, when combined into a soluble, fused MHC heterodimer:peptide complex, would allow manipulation of the immune system *in vivo*, in order to tolerize or anergize disease-associated activated T cells, thereby ameliorating the autoimmune disease.

Dissecting the functional role of individual peptides and peptide clusters in the interaction of a peptide ligand with an MHC molecule, and also in subsequent T cell recognition and reactivity, is a difficult undertaking due to the degeneracy of peptide binding to the MHC. Changes in T cell recognition or in the ability of an altered peptide to associate with MHC can be used to establish that a particular amino acid or group of amino acids comprises part of an MHC or T cell determinant. The interactions of altered peptides can be further assessed by competition with the parental peptide for presentation to a T cell, or through development of direct peptide-MHC binding assays. Changes to a peptide that do not involve MHC binding could well affect T cell recognition. For example, in a peptide, specific MHC contact points might only occur within a central core of a few consecutive or individual amino acids, whereas those amino acids involved in T cell recognition may include a completely different subset of residues.

In a preferred method, residues that alter T cell recognition are determined by substituting amino acids for each position in the peptide in question, and by assessing whether such change in residues alters the peptide's ability to associate with MHC (Allen *et al.*, *Nature* 327:713-15, 1987; Sette *et al.*, *Nature* 328:395-99, 1987; O'Sullivan *et al.*, *J. Immunol.* 147:2663-69, 1991; Evavold *et al.*, *J. Immunol.* 148:347-53, 1992; Jorgensen *et al.*, *Annu. Rev. Immunol.* 10:835-73, 1992; Hammer *et al.*, *Cell* 74:197-203, 1993; Evavold *et al.*, *Immunol. Today* 14:602-9, 1993; Hammer *et al.*, *Proc. Natl. Acad. Sci. USA* 91:4456-60, 1994; and Reich et al, *J. Immunol.* 154:2279-88, 1994). One method would involve generating a panel of altered peptides wherein individual or groups of amino acid residues are substituted with conservative, semi-conservative or non-conservative residues. A preferred variant of this method is an alanine scan (Ala scan) where a series of synthetic peptides are synthesized wherein each individual amino acid is substituted with L-alanine (L-Ala scan). Alanine is the amino acid of choice because it is found in all positions (buried and exposed), in secondary structure, it does not impose steric hindrances, or add additional hydrogen bonds or hydrophobic side chains. Alanine substitutions can be done independently or in clusters depending on the information

desired. Where the information pertains to specific residues involved in binding, each residue in the peptide under investigation can be converted to alanine and the binding affinity compared to the unsubstituted peptide. Additional structural and conformational information regarding each residue and the peptide as a whole can be gained, for example, by synthesizing a series of analogs wherein each residue is substituted with a D-amino acid such as D-alanine (D-Ala scan) (Galantino *et al.*, in Smith, J. and Rivier, J. (eds.), *Peptides Chemistry and Biology (Proceedings of the Twelfth American Peptide Symposium)*, ESCOM, Leiden, 1992, pp. 404-05). Essential residues can be identified, and nonessential residues targeted for modification, deletion or replacement by other residues that may enhance a desired quality (Cunningham and Wells, *Science* 244:1081-1085, 1989; Cunningham and Wells, *Natl. Acad. Sci. USA*, 88:3407-3411, 1991; Ehrlich *et al.*, *J. Biol. Chem.* 267:11606-11, 1992; Zhang *et al.*, *Proc. Natl. Acad. Sci. USA* 90:4446-50, 1993; see also "Molecular Design and Modeling: Concepts and Applications Part A Proteins, Peptides, and Enzymes," *Methods in Enzymology*, Vol. 202, Langone (ed.), Academic Press, San Diego, CA, 1991).

Truncated peptides can be generated from the altered or unaltered peptides by synthesizing peptides wherein amino acid residues are truncated from the N- or C-terminus to determine the shortest active peptide, or between the N- and C-terminus to determine the shortest active sequence. Such peptides could be specifically developed to stimulate a response when joined to a particular MHC to form a peptide ligand to induce anergy in appropriate T cells *in vivo* or *in vitro*.

Analysis of MHC class II heterodimer:peptide complexes

The physical and biological properties of the soluble, fused MHC heterodimer:peptide complexes may be assessed in a number of ways. Mass spectral analysis methods such as electrospray and Matrix-Assisted Laser Desorption/Ionization Time Of Flight mass spectrometry (MALDI TOF) analysis are routinely used in the art to provide such information as molecular weight and confirm disulfide bond formation. FACs analysis can be used to determine proper folding of the single chain complex.

An ELISA (Enzyme-linked Immunosorbent Assay) can be used to measure concentration and confirm correct folding of the soluble, fused MHC heterodimer:peptide complexes. This assay can be used with either whole cells;

solubilized MHC, removed from the cell surface; or free soluble, fused MHC heterodimer:peptide complexes of the current invention. In an exemplary ELISA, an antibody that detects the recombinant MHC haplotype is coated onto wells of a microtiter plate. In a preferred embodiment, the antibody is L243, a monoclonal antibody that recognizes only correctly folded HLA-DR MHC dimers. One of skill in the art will recognize that other MHC Class II-specific antibodies are known and available. Alternatively, there are numerous routine techniques and methodologies in the field for producing antibodies (for example, Hurrell, J.G.R. (ed)., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press Inc., Boca Raton, FL, 1982), if an appropriate antibody for a particular haplotype does not exist. Anti-MHC Class II antibodies can also be used to purify Class II molecules through techniques such as affinity chromatography, or as a marker reagent to detect the presence of Class II molecules on cells or in solution. Such antibodies are also useful for Western analysis or immunoblotting, particularly of purified cell-secreted material. Polyclonal, affinity purified polyclonal, monoclonal and single chain antibodies are suitable for use in this regard. In addition, proteolytic and recombinant fragments and epitope binding domains can be used herein. Chimeric, humanized, veneered, CDR-replaced, reshaped or other recombinant whole or partial antibodies are also suitable.

In the ELISA format, bound MHC molecules can be detected using an antibody or other binding moiety capable of binding MHC molecules. This binding moiety or antibody may be tagged with a detectable label, or may be detected using a detectably labeled secondary antibody or binding reagent. Detectable labels or tags are known in the art, and include fluorescent, colorimetric and radiolabels, for instance.

Other assay strategies can incorporate specific T-cell receptors to screen for their corresponding MHC-peptide complexes, which can be done either *in vitro* or *in vivo*. For example, an *in vitro* anergy assay determines if non-responsiveness has been induced in the T cells being tested. Briefly, an MHC molecule containing antigenic peptide in the peptide binding groove can be mixed with responder cells, preferably peripheral blood mononuclear cells (PBMN) (a heterogeneous population including B and T lymphocytes, monocytes and dendritic cells), PBMNC lymphocytes, freshly isolated T lymphocytes, *in vivo* primed splenocytes, cultured T cells, or established T cell lines or

clones. Responder cells from mammals immunized with, or having a demonstrable cellular immune response to, the antigenic peptide are particularly preferred.

Subsequently, these responder cells are combined with stimulator cells (antigen presenting cells; APCs) that have been pulsed or primed with the same antigenic peptide. In a preferred embodiment, the stimulator cells are antigenic peptide-presenting cells, such as PBMNCs, PBMNCs that have been depleted of lymphocytes, appropriate antigenic peptide-presenting cell lines or clones (such as EBV-transformed B cells), EBV transformed autologous and non-autologous PMNCs, genetically engineered antigen presenting cells, such as mouse L cells or bare lymphocyte cells BLS-1, in particular, DRB1*0401, DRB1*0404 and DRB1*0301 (Kovats et al, *J. Exp. Med.* 179:2017-22, 1994), or *in vivo* or *in vitro* primed or pulsed splenocytes. Stimulator cells from mammals immunized with, or having a demonstrable cellular immune response to, the antigenic peptide are particularly preferred. For certain assay formats, it is preferred to inhibit the proliferation of stimulator cells prior to mixing with responder cells. This inhibition may be achieved by exposure to gamma irradiation or to an anti-mitotic agent, such as mitomycin C, for instance. Appropriate negative controls are also included (nothing; syngeneic APC; experimental peptide; APC + Peptide; MHC:peptide complex; control peptide +/- APC). Further, to assure that non-responsiveness represents anergy, the proliferation assay may be set up in duplicate, +/- recombinant IL-2 since it has been demonstrated that IL-2, can rescue anergized cells.

After an approximately 72 hour incubation, the activation of responder cells in response to the stimulator cells is measured. In a preferred embodiment, responder cell activation is determined by measuring proliferation using ^{3}H-thymidine uptake (Crowley *et al.*, *J. Immunol. Meth.* 133:55-66, 1990). Alternatively, responder cell activation can be measured by the production of cytokines, such as IL-2, or by determining the presence of responder cell-specific, and particularly T cell-specific, activation markers. Cytokine production can be assayed by testing the ability of the stimulator + responder cell culture supernatant to stimulate growth of cytokine-dependent cells. Responder cell- or T cell-specific activation markers may be detected using antibodies specific for such markers.

Preferably, the soluble, fused MHC heterodimer:peptide complex induces non-responsiveness (for example, anergy) in the antigenic peptide-reactive responder

cells. In addition to soluble, fused MHC heterodimer:peptide complex recognition, responder cell activation requires the involvement of co-receptors on the stimulator cell (the APC) that have been stimulated with co-stimulatory molecules. By blocking or eliminating stimulation of such co-receptors (for instance, by exposing responder cells to purified soluble, fused MHC heterodimer:peptide complex, by blocking with anti-receptor or anti-ligand antibodies, or by "knocking out" the gene(s) encoding such receptors), responder cells can be rendered non-responsive to antigen or to soluble, fused MHC heterodimer:peptide complex.

In a preferred embodiment, responder cells are obtained from a source manifesting an autoimmune disease or syndrome. Alternatively, autoantigen-reactive T cell clones or lines are preferred responder cells. In another preferred embodiment, stimulator cells are obtained from a source manifesting an autoimmune disease or syndrome. Alternatively, APC cell lines or clones that are able to appropriately process and/or present autoantigen to responder cells are preferred stimulator cells. In a particularly preferred embodiment, responder and stimulator cells are obtained from a source with diabetes or multiple sclerosis.

At this point, the responder T cells can be selectively amplified and/or stimulated, thereby producing a subset of T cells that are specific for the antigenic peptide. For instance, antigenic peptide-reactive responder cells may be selected by flow cytometry, and particularly by fluorescence activated cell sorting. This subset of responder cells can be maintained by repetitive stimulation with APCs presenting the same antigenic peptide. Alternatively, responder cell clones or lines can be established from this responder cell subset. Further, this subset of responder cells can be used to map epitopes of the antigenic peptide and the protein from which it is derived.

Other methods to assess the biological activity of the soluble, fused MHC heterodimer:peptide complexes are known in the art and can be used herein, such as using a microphysiometer, to measure production of acidic metabolites in T cells following interaction with antigenic peptide. Other assay methods include competition assays, comparing soluble, fused MHC heterodimer:complex response with that to the normal antigen. Also measurement production of such indicators as cytokines or γ interferon can provide an indication of complex response.

Animal models of autoimmune disease

Similar assays and methods can be developed for and used in animal models. For instance, the therapeutic effect of a pharmaceutical composition of the single chain molecule or multimer or a polynucleotide encoding the single chain molecule or multimer can be tested *in vivo* in a number of animal models of HLA-DR-associated autoimmune disease. These diseases include, but are not limited to, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, pernicious anemia, rheumatoid arthritis, and systemic lupus erythematosus.

For example, NOD mice are a spontaneous model of IDDM. Treatment with the pharmaceutical compositions prior to or after onset of disease can be monitored by assay of urine glucose levels in the NOD mouse, as well as by *in vitro* T cell proliferation assays to assess reactivity to known autoantigens (*see, e.g.,* Kaufman *et al.*, *Nature* 366:69-72 (1993)) for example) . Alternatively, induced models of autoimmune disease, such as EAE, can be treated with pharmaceutical composition. Treatment in a preventive or intervention mode can be followed by monitoring the clinical symptoms of EAE.

Following is a description of several other animal models of HLA-DR-associated autoimmune disease which can be used to assay *in vivo* effects of the peptide. It will be obvious to one of skill in the art that other suitable animal models for autoimmune diseases can be utilized in a similar manner.

Systemic Lupus Erythematosus (SLE)

F_1 hybrids of autoimmune New Zealand black (NZB) mice and the phenotypically normal New Zealand White (NZW) mouse strain develop severe systemic autoimmune disease, more fulminant than that found in the parental NZB strain. These mice manifest several immune abnormalities, including antibodies to nuclear antigens and subsequent development of a fatal, immune complex-mediated glomerulonephritis with female predominance, remarkably similar to SLE in humans (Knight *et al., J. Exp. Med.* 147:1653 (1978)), which is incorporated hereby by reference.

In both the human and murine forms of the disease, a strong association with MHC gene products has been reported. HLA-DR2 and HLA-DR3 individuals are at a higher risk than the general population to develop SLE (Reinertsen *et al., N. Engl. J.*

Med. 299:515 (1970)), while in NZB/W F_1 mice (H-$2^{d/u}$), a gene linked to the h-2^u haplotype derived from the NZW parent contributes to the development of the lupus-like nephritis.

The effect of the invention can be measured by survival rates and by the progress of development of the symptoms, such as protenuria and appearance of anti-DNA antibodies.

Proteinuria can be measured by any method known to those of skill in the art, *e.g.* colorimetrically by the use of Uristix (Miles Laboratories, Inc., Elkhart, IN), giving an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 100 mg/dl; 3+, 300 mg/dl; and 4+, 1000 mg/dl.

The presence of anti-DNA specific antibodies in NZB/W F_1 mice can be determined by using a modification of a linked immunosorbent assay (ELISA) described by Zouali *et al., J. Immunol. Methods* 90:105 (1986)) which is incorporated herein by reference.

Myasthenia Gravis (MG)

Myasthenia gravis is one of several human autoimmune diseases linked to HLA-D (Safenberg, *et al., Tissue Antigens* 12:136 (1978); McDevitt *et al., Arth. Rheum.* 20:59 (1977)) which are incorporated herein by reference. In MG antibodies to the acetyl choline receptors (AcChoR) impair neuromuscular transmission by mediating loss of AcChoR in the postsynaptic membrane.

SJL/J female mice are a model system for human MG. In these animals, experimental autoimmune myasthenia gravis (EAMG) can be induced by immunizing the mice with soluble AcChoR protein from another species. Susceptibility to EAMG is linked in part to the MHC and has been mapped to the region within H-2 (Christadoss *et al., J. Immunol.* 123:2540 (1979)).

AcChoR protein can purified from *Torpedo californica* and assayed according to the method of Waldor *et al., Proc. Natl. Acad. Sci. USA* 80:2713 (1983), incorporated by reference. For example, emulsified AcChoR, 15 μg in complete Freund adjuvant, is injected intradermally among six sites on the back, the hind foot pads, and the base of the tail. Animals are reimmunized with this same regimen 4 weeks later.

Evaluation can be made by measurement of anti-AcChoR antibodies by any method known to those of skill in the art, *e.g.*, a microtiter ELISA assay as described in Waldor *et al., supra*. In an exemplary assay, the standard reagent volume is 50 µl per well. Reagents are usually incubated in the wells for 2 hr at RT. Five µg of AcChoR diluted in bicarbonate buffer, pH 9.6, is added to each well. After incubation with AcChoR, the plates are rinsed four times with a wash solution consisting of phosphate-buffer saline containing 0.05% Tween and 0.05% NaN_3. Mouse sera are diluted in 0.01M PBS (pH 7.2), 1.5 mfr $MgCl_2$, 2.0 mM 2-mercaptoethanol, 0.05% Tween-80, 0.05% NaN_3 (p-Tween buffer) and incubated on the plate. After the plate is washed, beta-galactosidase-conjugated sheep anti-mouse antibody diluted in P-Tween buffer is added to each well. After a final washing, the enzyme substrate, p-nitrophenylgalctopyranoside is added to the plate, and the degree of substrate catalysis is determined from the absorbance at 405 nm after 1 hr.

Anti-AcChoR antibodies are expected to be present in the mice immunized with AcChoR as compared to nonimmunized mice. Treatment with complex is expected to significantly reduce the titer of anti-AcChoR antibodies in the immunized mice.

The effect of treatment with the invention on clinical EAMG can also be assessed by any method known to those of skill in the art. Myasthenia symptoms include a characteristic hunched posture with drooping of the head and neck, exaggerated arching of the back, splayed limbs, abnormal walking, and difficulty in righting. Mild symptoms are present after a standard stress test, and should be ameliorated by administration of complex.

Rheumatoid Arthritis (RA)

In humans, susceptibility to rheumatoid arthritis is associated with HLA D/DR. The immune response in mice to native type II collagen has been used to establish an experimental model for arthritis with a number of histological and pathological features resembling human RA. Susceptibility to collagen-induced arthritis (CIA) in mice has been mapped to the H-2 I region, particularly the I-A subregion (Huse *et al., Fed. Proc.* 43:1820 (1984)).

Mice from a susceptible strain, DEA-1 can be caused to have CIA by treatment of the mice with native type II collagen, using the technique described in Wooley *et al.*, *J. Immunol.* 134:2366 (1985), incorporated herein by reference.

In another model adjuvant arthritis in rats is an experimental model for human arthritis, and a prototype of autoimmune arthritis triggered by bacterial antigens (Holoschitz *et al., Prospects of Immunology* (1986); Pearson, *Arthritis Rheum.* 7:80 (1964)). The disease is the result of a cell-mediated immune response, as evidenced by its transmissibility by a clone of T cells which were reactive against the adjuvant (MT); the target self-antigen in the disease, based upon studies with the same cloned cells, appears to be part(s) of a proteoglycan molecule of cartilage.

Adjuvant disease in rats is produced as described by Pearson *supra, i.e.,* by a single injection of Freund's adjuvant (killed tubercle bacilli or chemical fractions of it, mineral oil, and an emulsifying agent) given into several depot sites, preferably intracutaneously or into a paw or the base of the tail. The adjuvant is given in the absence of other antigens.

The effect of the invention treatment on manifestations of the disease can be monitored by any method known to those of skill in the art. These manifestations are histopathological, and include an acute and subacute synovitis with proliferation of synovial lining cells, predominantly a mononuclear infiltration of the articular and particular tissues, the invasion of bone and articular cartilage by connective tissue pannus, and periosteal new bone formation, especially adjacent to affected joints. In severe or chronic cases, destructive changes occur, as do fibrous or bony ankylosis. These histopathological symptoms are expected to appear in control animals at about 12 days after sensitization to the Freund's adjuvant.

Insulin Dependent Diabetes Mellitus (IDDM)

IDDM is observed as a consequence of the selective destruction of insulin-secreting cells within the Islets of Langerhans of the pancreas. Involvement of the immune system in this disease is suggested by morphologic evidence of early infiltration of the Islets by mononuclear cells, by the detection of anti-islet cell antibodies, by the high frequency of HLA-DR3 and -DR4 alleles in IDDM populations, and by clinical associations between IDDM and various autoimmune diseases. An animal model for

spontaneous IDDM and thyroiditis has been developed in the BB rat. As in humans, the rat disease is controlled in part by the genes encoding the MHC antigens, is characterized by islet infiltration, and is associated with the presence of anti-islet antibodies. The I-E equivalent class II MHC antigens appear to be involved in manifestation of the autoimmune diseases in the BB rat. Biotard *et al., Proc. Natl. Acad. Sci. USA* 82:6627 (1985).

In morphologic evaluation, insulitis is characterized by the presence of mononuclear inflammatory cells within the islets. Thyroiditis is characterized by focal interstitial lymphocytic infiltrate within the thyroid gland, as a minimum criterion. Most severe cases show diffuse extensive lymphocytic infiltrates, disruption of acini, fibrosis, and focal Hurthle call change. See Biotard *et al. supra.*

Treatment of the BB rats with the invention is expected to ameliorate or prevent the manifestation of the clinical and morphological symptoms associated with IDDM and thyroiditis.

In another model, the NOD mouse strain (H-2K^d D^b) is a murine model for autoimmune IDDM. The disease in these animals is characterized by anti-islet cell antibodies, severe insulitis, and evidence for autoimmune destruction of the beta-cells (Kanazawa, *et al., Diabetolooia* 27:113 (1984)). The disease can be passively transferred with lymphocytes and prevented by treatment with cyclosporin-A (Ikehara *et al., Proc. Natl. Acad. Sci. USA* 82:7743 (1985)); Mori *et al., Diabetolooia* 29:244 (1986). Untreated animals develop profound glucose intolerance and ketosis and succumb within weeks of the onset of the disease. Seventy to ninety percent of female and 20-30% of male animals develop diabetes within the first six months of life. Breeding studies have defined at least two genetic loci responsible for disease susceptibility, one of which maps to the MHC. Characterization of NOD Class II antigens at both the serologic and molecular level suggest that the susceptibility to autoimmune disease is linked to I-A_B (Acha-Orbea and McDevitt, *Proc. Natl. Acad. Sci. USA* 84:235 (1907)).

Treatment of Female NOD mice with complex is expected to lengthen the time before the onset of diabetes and/or to ameliorate or prevent the disease.

Experimental Allergic Encephalomyelitis (EAE)

Experimental allergic encephalomyelitis (EAE) is an induced autoimmune disease of the central nervous system which is a model for multiple sclerosis (MS). The disease can be induced in many species, including mice and rats.

The disease is characterized by the acute onset of paralysis. Perivascular infiltration by mononuclear cells in the CNS is observed in both mice and rats. Methods of inducing the disease, as well as symptomology, are reviewed in Aranson, *The Autoimmune Diseases* (Rose and Mackay, eds., 1985), and in Acha-Orbea *et al., Ann. Rev. Imm.* 7:377-405 (1989).

One of the genes mediating susceptibility is localized in the MHC class II region (Moore *et al., J. Immunol.* 124:1815-1820 (1980)). The best analyzed encephalitogenic protein is myelin basic protein (MBP), but other encephalitogenic antigens are found in the brain. The immunogenic epitopes have been mapped (*see*, Acha-Orbea *et al., supra.*). In the PL mouse strains (H-2^u) two encephalitogenic peptides in MBP have been characterized: MBP peptide p35-47 (MBP 35-47), and acetylated NSF p1-9 (MBP 1-9).

The effect of the invention on ameliorating disease symptoms in individuals in which EAE has been induced can be measured by survival rates, and by the progress of the development of symptoms.

Methods of making the complexes of the invention

Expression systems suitable for production of appropriate soluble, fused MHC heterodimer:peptide complexes are available and known in the art. Various prokaryotic, fungal, and eukaryotic host cells are suitable for expression of soluble, fused MHC heterodimer:peptide complexes.

Prokaryotes that are useful as host cells, according to the present invention, most frequently are represented by various strains of *Escherichia coli*. However, other microbial strains can also be used, such as bacilli, for example *Bacillus subtilis*, various species of *Pseudomonas*, or other bacterial strains.

According to the invention, the soluble, fused MHC heterodimer:peptide complexes are expressed from recombinantly engineered nucleotide sequences that encode the soluble, fused MHC heterodimer:peptide polypeptides by operably linking the

engineered nucleic acid coding sequence to signals that direct gene expression in prokaryotes. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it effects the transcription of the sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

The genes encoding the soluble, fused MHC heterodimer:peptide complexes may be inserted into an "expression vector," "cloning vector," or "vector," terms which are used interchangeably herein and usually refer to plasmids or other nucleic acid molecules that are able to replicate in a chosen host cell. Expression vectors may replicate autonomously, or they can replicate by being inserted into the genome of the host cell, by methods well known in the art. Vectors that replicate autonomously will have an origin of replication or autonomous replicating sequence (ARS) that is functional in the chosen host cell(s).

Plasmid vectors that contain replication sites and control sequences derived from a species compatible with the chosen host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from *E. coli* species by Bolivar *et al.*, *Gene* 2:95-113, 1977. Often, it is desirable for a vector to be usable in more than one host cell, e.g., in *E. coli* for cloning and construction, and in a Bacillus cell for expression.

The expression vectors typically contain a transcription unit or expression cassette that contains all the elements required for the expression of the DNA encoding the MHC molecule in the host cells. A typical expression cassette contains a promoter operably linked to the DNA sequence encoding a soluble, fused MHC heterodimer:peptide complex and a ribosome binding site. The promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function. In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient

termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from a different gene.

Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (lac) promoter systems (Change *et al.*, *Nature* 198:1056, 1977) and the tryptophan (trp) promoter system (Goeddel *et al.*, *Nucleic Acids Res.* 8:4057-74, 1980) and the lambda-derived P_L promoter and N-gene ribosome binding site (Shimatake *et al.*, *Nature* 292:128-32, 1981). Any available promoter system that functions in prokaryotes can be used.

Either constitutive or regulated promoters can be used in the present invention. Regulated promoters can be advantageous because the host cells can be grown to high densities before expression of the soluble, fused MHC heterodimer:peptide complexes is induced. High level expression of heterologous proteins slows cell growth in some situations. Regulated promoters especially suitable for use in *E. coli* include the bacteriophage lambda P_L promoter, the hybrid *trp-lac* promoter (Amann *et al.*, *Gene* 25:167-78 1983; and the bacteriophage T7 promoter.

For expression of soluble, fused MHC heterodimer:peptide complexes in prokaryotic cells other than *E. coli*, a promoter that functions in the particular prokaryotic species is required. Such promoters can be obtained from genes that have been cloned from the species, or heterologous promoters can be used. For example, the hybrid trp-lac promoter functions in Bacillus in addition to *E. coli*.

A ribosome binding site (RBS) is also necessary for expression of soluble, fused MHC heterodimer:peptide complexes in prokaryotes. An RBS in *E. coli*, for example, consists of a nucleotide sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine and Dalgarno, *Nature,* 254:34-40, 1975; Steitz, In Biological regulation and development: *Gene expression* (ed. R.F. Goldberger), vol. 1, p. 349, 1979, Plenum Publishing, NY).

Translational coupling may be used to enhance expression. The strategy uses a short upstream open reading frame derived from a highly expressed gene native to the translational system, which is placed downstream of the promoter, and a ribosome binding site followed after a few amino acid codons by a termination codon. Just prior to

the termination codon is a second ribosome binding site, and following the termination codon is a start codon for the initiation of translation. The system dissolves secondary structure in the RNA, allowing for the efficient initiation of translation. See Squires, et. al., *J. Biol. Chem.* 263:16297-16302, 1988.

The soluble, fused MHC heterodimer:peptide complexes can be expressed intracellularly, or can be secreted from the cell. Intracellular expression often results in high yields. However, some of the protein may be in the form of insoluble inclusion bodies. Although some of the intracellularly produced MHC polypeptides of the present invention may be active upon being harvested following cell lysis, the amount of soluble, active MHC polypeptide may be increased by performing refolding procedures using methods known to those of skill in the art (*see, e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual Second Edition*, Cold Spring Harbor, NY, 1989.; Marston *et al.*, *Bio/Technology* 2:800-804, 1985; Schoner *et al.*, *Bio/Technology* 3:151-54, 1985). In one embodiment, for purification and refolding the cell pellet is lysed and refolded in urea-borate-DTT buffer followed by urea-borate buffer and reverse phase HPLC purification using either silica gel based Vydac (Hewlett Packard, Wilmington, DE) or polymer based Poros-R2 (PerSeptive Biosystems) resins, with bead size varying based on the scale of the culture and is described in further detail below. In one embodiment, e.g., for large scale refolding, the sample can be ultrafiltered into a urea-borate buffer to which is then added 0.2 μM to 1 mM copper sulfate, preferably 0.2 to 20 μM, after which folding occurs immediately.

More than one MHC:peptide complex may be expressed in a single prokaryotic cell by placing multiple transcriptional cassettes in a single expression vector, or by utilizing different selectable markers for each of the expression vectors which are employed in the cloning strategy.

A second approach for expressing the MHC:peptide complexes of the invention is to cause the polypeptides to be secreted from the cell, either into the periplasm or into the extracellular medium. The DNA sequence encoding the MHC polypeptide is linked to a cleavable signal peptide sequence. The signal sequence directs translocation of the MHC:peptide complex through the cell membrane. An example of a suitable vector for use in *E. coli* that contains a promoter-signal sequence unit is pTA1S29, which has the *E. coli* phoA promoter and signal sequence *see*, e.g., Sambrook

et al., *supra*; Oka *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7212-16, 1985; Talmadge *et al.*, *Proc. Natl. Acad. Sci. USA* 77:39892, 1980; Takahara *et al.*, *J. Biol. Chem.* 260: 2670-74, 1985). Once again, multiple polypeptides can be expressed in a single cell for periplasmic association.

The MHC:peptide complexes of the invention can also be produced as fusion proteins. This approach often results in high yields, because normal prokaryotic control sequences direct transcription and translation. In *E. coli*, lacZ fusions are often used to express heterologous proteins. Suitable vectors are readily available, such as the pUR, pEX, and pMR100 series (*see, e.g.*, Sambrook *et al.*, *supra*). For certain applications, it may be desirable to cleave the non-MHC amino acids from the fusion protein after purification. This can be accomplished by any of several methods known in the art, including cleavage by cyanogen bromide, a protease, or by Factor X, (see, e.g. Sambrook *et al.*, *supra.*; Goeddel *et al.*, *Proc. Natl. Acad. Sci. USA* 76:106-10, 1979; Nagai *et al.*, *Nature* 309:810-l2, 1984; Sung *et al.*, *Proc. Natl. Acad. Sci. USA* 83:561-65, 1986). Cleavage sites can be engineered into the gene for the fusion protein at the desired point of cleavage.

Foreign genes, such as soluble, fused MHC heterodimer:peptide complexes, can be expressed in *E. coli* as fusions with binding partners, such as glutathione-S-transferase (GST), maltose binding protein, or thioredoxin. These binding partners are highly translated and can be used to overcome inefficient initiation of translation of eukaryotic messages in *E. coli*. Fusion to such binding partner can result in high-level expression, and the binding partner is easily purified and then excised from the protein of interest. Such expression systems are available from numerous sources, such as Invitrogen Inc. (San Diego, CA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ).

A method for obtaining recombinant proteins from *E. coli* which maintains the integrity of their N-termini has been described by Miller *et al. Biotechnology* 7:698-704 (1989). In this system, the gene of interest is produced as a C-terminal fusion to the first 76 residues of the yeast ubiquitin gene containing a peptidase cleavage site. Cleavage at the junction of the two moieties results in production of a protein having an intact authentic N-terminal reside.

The vectors containing the nucleic acids that code for the soluble, fused MHC heterodimer:peptide complexes are transformed into prokaryotic host cells for expression. "Transformation" refers to the introduction of vectors containing the nucleic acids of interest directly into host cells by well known methods. The particular procedure used to introduce the genetic material into the host cell for expression of the soluble, fused MHC heterodimer:peptide complex is not particularly critical. Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. It is only necessary that the, particular host cell utilized be capable of expressing the gene.

Transformation methods, which vary depending on the type of the prokaryotic host cell, include electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, or other substances; microprojectile bombardment; infection (where the vector is an infectious agent) ; and other methods. See, generally, Sambrook *et al, supra*, and Ausubel *et al.*, (eds.) *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987. Reference to cells into which the nucleic acids described above have been introduced is meant to also include the progeny of such cells. Transformed prokaryotic cells that contain expression vectors for soluble, fused MHC heterodimer:peptide complexes are also included in the invention.

After standard transfection or transformation methods are used to produce prokaryotic cell lines that express large quantities of the soluble, fused MHC heterodimer:peptide complex polypeptide, the polypeptide is then purified using standard techniques. *See, e.g.,* Colley *et al., J. Chem.* 64:17619-22, 1989; and *Methods in Enzymology*, "Guide to Protein Purification", Deutscher, ed., Vol. 182 (1990). The recombinant cells are grown and the soluble, fused MHC heterodimer:peptide complex is expressed. The purification protocol will depend upon whether the soluble, fused MHC heterodimer:peptide complex is expressed intracellularly, into the periplasm, or secreted from the cell. For intracellular expression, the cells are harvested, lysed, and the polypeptide is recovered from the cell lysate (Sambrook *et al., supra*). Periplasmic MHC polypeptide is released from the periplasm by standard techniques (Sambrook *et al., supra*). If the MHC polypeptide is secreted from the cells, the culture medium is harvested for purification of the secreted protein. The medium is typically clarified by centrifugation or filtration to remove cells and cell debris.

The MHC polypeptides can be concentrated by adsorption to any suitable resin (such as, for example, CDP-Sepharose, Asialoprothrombin-Sepharose 4B, or Q Sepharose), or by use of ammonium sulfate fractionation, polyethylene glycol precipitation, or by ultrafiltration. Other means known in the art may be equally suitable.

Further purification of the MHC polypeptides can be accomplished by standard techniques, for example, affinity chromatography, ion exchange chromatography, sizing chromatography, reverse phase HPLC, or other protein purification techniques used to obtain homogeneity. The purified proteins are then used to produce pharmaceutical compositions.

For secretion of a polypeptide or protein of interest, recombinant nucleic acid constructs of the invention may include sequences that encode signal sequences or other sequences that direct secretion. Secretory signal sequences, also called leader sequences, prepro sequences and/or pre sequences, are amino acid sequences that play a role in secretion of mature polypeptides or proteins from a cell. Such sequences are characterized by a core of hydrophobic amino acids and are typically (but not exclusively) found at the amino termini of newly synthesized proteins. The secretory signal sequence may be that of the protein of interest, or may be derived from another secreted protein (e.g., t-PA, a preferred mammalian secretory leader) or synthesized de novo. The secretory signal sequence is joined to the DNA sequence encoding a protein of the present invention in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830). Very often the secretory peptide is cleaved from the mature protein during secretion. Such secretory peptides contain processing sites that allow cleavage of the secretory peptide from the mature protein as it passes through the secretory pathway. An example of such a processing site is a dibasic cleavage site, such as that recognized by the *Saccharomyces cerevisiae* KEX2 gene or a Lys-Arg processing site. Processing sites may be encoded within the secretory peptide or may be added to the peptide by, for example, *in vitro* mutagenesis.

Secretory signals include the α factor signal sequence (prepro sequence: Kurjan and Herskowitz, *Cell* 3:933-943, 1982; Kurjan *et al.*, U.S. Patent No. 4,546,082;

Brake, EP 116, 201), the *PHO5* signal sequence (Beck *et al.*, WO 86/00637) , the BAR1 secretory signal sequence (MacKay *et al.*, U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the *SUC2* signal sequence (Carlsen *et al.*, *Molecular and Cellular Biology* 3: 439-447 , 1983) , the a-l-antitrypsin signal sequence (Kurachi *et al.*, *Proc. Natl. Acad. Sci. USA* 78: 6826-6830, 1981) , the a-2 plasmin inhibitor signal sequence (Tone *et al.*, *J. Biochem. (Tokyo)* 102: 1033-1042, 1987) and the tissue plasminogen activator signal sequence (Pennica *et al.*, *Nature* 301: 214-221, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (*European Journal of Biochemistry* 133: 17-21, 1983; *Journal of Molecular Biology* 184: 99-105, 1985; *Nucleic Acids Research* 14: 4683-4690; 1986). Another signal sequence is the synthetic signal LaC212 spx (1-47). ERLE described in WO 90/10075.

Secretory signal sequences may be used singly or may be combined. For example, a first secretory signal sequence may be used in combination with a sequence encoding the third domain of barrier (described in U.S. Patent No. 5,037,243, which is incorporated by reference herein in its entirety). The third domain of barrier may be positioned in proper reading frame 3' of the DNA segment of interest or 5' to the DNA segment and in proper reading frame with both the secretory signal sequence and a DNA segment of interest.

The choice of suitable promoters, terminators and secretory signals for all expression systems, is well within the level of ordinary skill in the art. Methods for expressing cloned genes in *Saccharomyces cerevisiae* are generally known in the art (see, "Gene Expression Technology," *Methods in Enzymology*, Vol. 185, Goeddel (ed.), Academic Press, San Diego, CA, 1990 and "Guide to Yeast Genetics and Molecular Biology, "*Methods in Enzymology*, Guthrie and Fink (eds.), Academic Press, San Diego, CA, 1991; which are incorporated herein by reference). Proteins of the present invention can also be expressed in filamentous fungi, for example, strains of the fungi *Aspergillus* (McKnight *et al.*, U.S. Patent No. 4,935,349, which is incorporated herein by reference). Expression of cloned genes in cultured mammalian cells and in *E. coli*, for example, is discussed in detail in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual. Second Edition*, Cold Spring Harbor, NY, 1989; which is incorporated herein by reference). As would be evident to one skilled in the art, one could express the proteins

of the instant invention in other host cells such as avian, insect and plant cells using regulatory sequences, vectors and methods well established in the literature.

In yeast, suitable yeast vectors for use in the present invention include YRp7 (Struhl *et al.*, *Proc. Natl. Acad. Sci. USA* 76:1035-1039, 1978), YEpl3 (Broach *et al.*, *Gene* 8: 121-133, 1979), POT vectors (Kawasaki et al, U.S. Patent No. 4,931,373, which is incorporated by reference herein), pJDB249 and pJDB219 (Beggs, *Nature* 275:104-108, 1978) and derivatives thereof. Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman *et al.*, *J. Biol. Chem.* 255: 12073-12080, 1980; Alber and Kawasaki, *J. Mol. Appl. Genet.* 1: 419-434, 1982; Kawasaki, U.S. Patent No. 4,599,311) or alcohol dehydrogenase genes (Young *et al.*, in *Genetic Engineering of Microorganisms for Chemicals*, Hollaender *et al.*, (eds.), p. 355, Plenum, New York, 1982; Ammerer, *Meth. Enzymol.* 101: 192-201, 1983). Other promoters are the TPI1 promoter (Kawasaki, U.S. Patent No. 4,599,311, 1986) and the ADH2-4^c promoter (Russell *et al.*, *Nature* 304: 652-654, 1983; Irani and Kilgore, U.S. Patent Application Serial No. 07/784,653, CA 1,304,020 and EP 284 044, which are incorporated herein by reference). The expression units may also include a transcriptional terminator such as the TPI1 terminator (Alber and Kawasaki, *ibid.*).

Yeast cells, particularly cells of the genus *Pichia* or *Saccharomyces*, are a preferred host for use in producing compound of the current invention. Methods for transforming yeast cells with exogenous DNA and producing recombinant proteins therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075, which are incorporated herein by reference. Transformed cells are selected by phenotype determined by a selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine) . A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. A preferred secretory signal sequence for use in yeast is that of the *S. cerevisiae* MFα1 gene (Brake, *ibid.*; Kurjan *et al.*, U.S. Patent No. 4,546,082) . Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No. 4,615,974; and

Bitter, U.S. Patent No. 4,977,092, which are incorporated herein by reference) and alcohol dehydrogenase genes. See also U.S. Patent Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454, which are incorporated herein by reference. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459-65, 1986; Cregg, U.S. Patent No. 4,882,279; and Stroman *et al.*, U.S. Patent No. 4,879,231.

Other fungal cells are also suitable as host cells. For example, *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349, which is incorporated herein by reference. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228, which is incorporated herein by reference. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533, which is incorporated herein by reference.

Host cells containing DNA constructs of the present invention are then cultured to produce the heterologous proteins. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the particular host cells. A variety of suitable media are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals and growth factors. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by a selectable marker on the DNA construct or co-transfected with the DNA construct.

Yeast cells, for example, are preferably cultured in a chemically defined medium, comprising a non-amino acid nitrogen source, inorganic salts, vitamins and essential amino acid supplements. The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, preferably at pH 6.5. Methods for maintaining a stable pH include buffering and constant pH control, preferably through the addition of sodium hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO). Yeast cells having a defect in a gene required for asparagine-linked glycosylation are preferably grown in a medium containing an osmotic

stabilizer. A preferred osmotic stabilizer is sorbitol supplemented into the medium at a concentration between 0.1 M and 1.5 M, preferably at 0.5 M or 1.0 M. Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media. Selection of a medium appropriate for the particular host cell used is within the level of ordinary skill in the art.

Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981; Graham and Van der Eb; *Virology* 52:456, 1973) , electroporation (Neumann *et al.*, *EMBO J.* 1:841-45, 1982) and DEAE-dextran mediated 'transfection (Ausubel *et al.*, (eds), *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987), which are incorporated herein by reference. Cationic lipid transfection using commercially available reagents, including the Boehringer Mannheim TRANSFECTION-REAGENT (N-[1- (2,3-dioleoyloxy)propyl] -N,N,N-trimethyl ammoniummethylsulfate; Boehringer Mannheim, Indianapolis, IN) or LIPOFECTIN reagent (N-[l-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleoyl phosphatidylethanolamine; GIBCO-BRL, Gaithersburg, MD) using the manufacturer-supplied directions, may also be used. A preferred mammalian expression plasmid is Zem229R (deposited under the terms of the Budapest Treaty with American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD on September 28, 1993 as an *E. coli* HB101 transformant and assigned Accession Number 69447) . The production of recombinant proteins in cultured mammalian cells is disclosed, for example, by Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134, which are incorporated herein by reference. Preferred cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), DG44, and 293 (ATCC No. CRL 1573; Graham *et al.*, *J. Gen. Virol.* 36:59-72, 1977) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include

those from metallothionein genes (U.S. Patents Nos. 4,579,821 and 4,601,978, which are incorporated herein by reference) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants." Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

The soluble, fused MHC:peptide complexes of the present invention can be purified by first isolating the polypeptides from the cells followed by conventional purification methods, such as by ion-exchange and partition chromatography as described by, for example, Coy *et al.* (Peptides Structure and Function, Pierce Chemical Company, Rockford, IL, pp 369-72, 1983) or by reverse-phase chromatography as described, for example, by Andreu and Merrifield (*Eur. J. Biochem.* 164: 585-90, 1987), or by HPLC as described, for example, by Kofod *et al.* (*Int. J. Peptide and Protein Res.* 32.: 436-40, 1988). Additional purification can be achieved by additional conventional purification means, such as liquid chromatography, gradient centrifugation, and gel electrophoresis, among others. Methods of protein purification are known in the art (see generally, Scopes, R., *Protein Purification*, Springer-Verlag, NY, 1982, which is incorporated by reference herein) and can be applied to the purification of the recombinant polypeptides described herein. Soluble, fused MHC heterodimer:peptide complexes of at least about 50% purity are preferred, at least about 70-80% purity more preferred, and about 95-99% or more purity most preferred, particularly for pharmaceutical uses. Once purified, either

partially or to homogeneity, as desired, the soluble, fused MHC heterodimer:peptide complexes may then be used diagnostically or therapeutically, as further described below.

Methods of using the MHC class II heterodimer:peptide complexes

The soluble, fused MHC heterodimer:peptide complexes of the present invention may be used within methods for down-regulating parts of the immune system that are reactive in autoimmune diseases. The soluble, fused MHC heterodimer:peptide complexes of the present invention are contemplated to be advantageous for use as immunotherapeutics to induce immunological tolerance or nonresponsiveness (anergy) in patients predisposed to mount or already mounting an immune response those particular autoantigens. A patient having or predisposed to a particular autoimmune disease is identified and MHC type is determined by methods known in the art. The patient's T cells can be examined *in vitro* to determine autoantigenic peptide(s) recognized by the patient's autoreactive T cells using complexes and methods described herein. The patient can then be treated with complexes of the invention. Such methods will generally include administering soluble, fused MHC heterodimer:peptide complex in an amount sufficient to lengthen the time period before onset of the autoimmune disease and/or to ameliorate or prevent that disease. Soluble, fused MHC heterodimer:peptide complexes of the present invention are therefore contemplated to be advantageous for use in both therapeutic and diagnostic applications related to auto immune diseases.

Kits can also be supplied for therapeutic or diagnostic uses. Thus, the subject composition of the present invention may be provided, usually in a lyophilized form, in a container. The soluble, fused MHC heterodimer:peptide complex is included in the kits with instructions for use, and optionally with buffers, stabilizers, biocides, and inert proteins. Generally, these optional materials will be present at less than about 5% by weight, based on the amount of soluble, fused MHC heterodimer:peptide complex, and will usually be present in a total amount of at least about 0.001% by weight, based on the soluble, fused MHC heterodimer:peptide complex concentration. It may be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient may be present in from about 1 to 99% weight of the total composition.

Within one aspect of the present invention, soluble, fused MHC heterodimer:peptide complexes are utilized to prepare antibodies for diagnostic or

therapeutic uses. As used herein, the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as $F(ab')_2$ and Fab fragments, as well as recombinantly produced binding partners. These binding partners incorporate the variable or CDR regions from a gene which encodes a specifically binding antibody. The affinity of a monoclonal antibody or binding partner may be readily determined by one of ordinary skill in the art (see, Scatchard, *Ann. NY Acad. Sci.* 51: 660-72, 1949)

Methods for preparing polyclonal and monoclonal antibodies have been well described in the literature (*see*, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press, Ind., Boca Raton, FL, 1982, which is incorporated herein by reference) . As would be evident to one of ordinary skill in the art, polyclonal antibodies may be generated from a variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, for example. The immunogenicity of the soluble, fused MHC heterodimer:peptide complexes may be increased through the use of an adjuvant, such as Freund's complete or incomplete adjuvant. A variety of assays known to those skilled in the art may be utilized to detect antibodies which specifically bind to a soluble, fused MHC heterodimer:peptide complex. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual,* Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-immunoprecipitations, enzyme-linked immuno-sorbent assays, dot blot assays, inhibition or competition assays, and sandwich assays.

Additional techniques for the preparation of monoclonal antibodies may be utilized to construct and express recombinant monoclonal antibodies. Briefly, mRNA is isolated from a B cell population and used to create heavy and light chain immunoglobulin cDNA expression libraries in a suitable vector such as the λIMMUNOZAP(H) and λIMMUNOZAP(L) vectors, which may be obtained from Stratagene Cloning Systems (La Jolla, CA). These vectors are then screened individually or are co-expressed to form Fab fragments or antibodies (Huse *et al.*, *Science* 246 1275-81, 1989; Sastry *et al.*, *Proc. Natl. Acad. Sci. USA 86*: 5728-32, 1989). Positive plaques

are subsequently converted to a non-lytic plasmid which allows high level expression of monoclonal antibody fragments in *E. coli*.

Antibodies of the present invention may be produced by immunizing an animal selected from a wide variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats, with a recombinant soluble, fused MHC heterodimer:peptide complex. Serum from such animals are a source of polyclonal antibodies. Alternatively antibody producing cells obtained from the immunized animals are immortalized and screened. As the generation of human monoclonal antibodies to a human antigen, such as a soluble, fused MHC heterodimer:peptide complex, may be difficult with conventional immortalization techniques, it may be desirable to first make non-human antibodies. Using recombinant DNA techniques, the antigen binding regions of the non-human antibody is transferred to the corresponding site of a human antibody coding region to produce a substantially human antibody molecules. Such methods are generally known in the art and are described in, for example, U.S. Patent No. 4,816,397, and EP publications 173,494 and 239,400, which are incorporated herein by reference.

In another aspect of the invention, the soluble, fused MHC heterodimer:peptide complexes can be used to clone T cells which have specific receptors for the soluble, fused MHC heterodimer:peptide complex. Once the soluble, fused MHC heterodimer:peptide complex-specific T cells are isolated and cloned using techniques generally available to the skilled artisan, the T cells or membrane preparations thereof can be used to immunize animals to produce antibodies to the soluble, fused MHC heterodimer:peptide complex receptors on T cells. The antibodies can be polyclonal or monoclonal. If polyclonal, the antibodies can be murine, lagomorph, equine, ovine, or from a variety of other mammals. Monoclonal antibodies will typically be murine in origin, produced according to known techniques, or human, as described above, or combinations thereof, as in chimeric or humanized antibodies. The anti-soluble, fused MHC heterodimer:peptide complex receptor antibodies thus obtained can then be administered to patients to reduce or eliminate T cell subpopulations that display such receptor. This T-cell population recognizes and participates in the immunological destruction of cells bearing the autoantigenic peptide in an individual predisposed to or already suffering from a disease, such as an autoimmune disease related to the autoantigenic peptide.

The coupling of antibodies to solid supports and their use in purification of proteins is well known in the literature (*see,* for example, *Methods in Molecular Biology. Vol. 1*, Walker (Ed.), Humana Press, New Jersey, 1984, which is incorporated by reference herein in its entirety). Antibodies of the present invention may be used as a marker reagent to detect the presence of MHC heterodimer:peptide complexes on cells or in solution. Such antibodies are also useful for Western analysis or immunoblotting, particularly of purified cell-secreted material. Polyclonal, affinity purified polyclonal, monoclonal and single chain antibodies are suitable for use in this regard. In addition, proteolytic and recombinant fragments and epitope binding domains can be used herein. Chimeric, humanized, veneered, CDR-replaced, reshaped or other recombinant whole or partial antibodies are also suitable.

Pharmaceutical compositions

Pharmaceutically acceptable carriers are determined in part by the particular composition being administered (*e.g.*, nucleic acid, protein) as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g.*, *Remington's Pharmaceutical Sciences* (17^{th} ed., 1989). Administration can be in any convenient manner, *e.g.*, by injection, oral administration, inhalation, or transdermal application.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the packaged nucleic acid or polypeptide suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents, dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, *e.g.*, sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia

emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, they can be "nebulized") to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration and intravenous administration are the preferred methods of administration. The formulations of commends can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

The polypeptides of the invention are administered prophylactically or to an individual already suffering from the disease. The compositions are administered to a patient in an amount sufficient to elicit an effective immune response. An amount adequate to accomplish this is defined as "therapeutically effective dose" or "immunogenically effective dose." Amounts effective for this use will depend on, *e.g.*, the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range for the initial immunization dose (that is for therapeutic or prophylactic administration) from about 0.01 mg to about 50 mg per 70 kilogram patient, more commonly from about 0.5-1 mg to about 10-15 mg per 70 kg of body weight. Boosting dosages are typically from about 0.01 mg to about 50

mg of peptide, more commonly about 0.5-1 mg to about 10-15 mg, using a boosting regimen over weeks to months depending upon the patient's response and condition. A suitable protocol would include injection at time 0, 2, 6, 8, 10 and 14 weeks, followed by booster injections at 24 and 28 weeks. Booster injections can be from one, two, three, four, five or more. Initial and booster injection amounts and timing are determined based on the judgment of the physician and the antigen being administered. In one embodiment, the initial and booster dose is 1.3 mg, 4 mg, or 13 mg, administered via intramuscular injection, with at least one and up to 3 booster injections at 8 week intervals, or at least one and up to 4 booster injections at 6 week intervals.

The therapeutic methods of the present invention may involve oral tolerance (Weiner *et al.*, *Nature* 376: 177-80, 1995), or intravenous tolerance, for example. Tolerance can be induced in mammals, although conditions for inducing such tolerance will vary according to a variety of factors. To induce immunological tolerance in an adult susceptible to or already suffering from an autoantigen-related disease such as IDDM, the precise amounts and frequency of administration will also vary. For instance for adults about 20-80 μg/kg can be administered by a variety of routes, such as parenterally, orally, by aerosols, intradermal injection, and the like. For neonates, tolerance can be induced by parenteral injection or more conveniently by oral administration in an appropriate formulation. The precise amount administrated, and the mode and frequency of dosages, will vary.

The soluble, fused MHC heterodimer:peptide complexes will typically be more tolerogenic when administered in a soluble form, rather than in an aggregated or particulate form. Persistence of a soluble, fused MHC heterodimer:peptide complex of the invention is generally needed to maintain tolerance in an adult, and thus may require more frequent administration of the complex, or its administration in a form which extends the half-life of the complex. See for example, Sun *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 10795-99, 1994.

Within another aspect of the invention, a pharmaceutical composition is provided which comprises a soluble, fused MHC heterodimer:peptide complex of the present invention contained in a pharmaceutically acceptable carrier or vehicle for parenteral, topical, oral, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment, according to conventional methods. The

composition may typically be in a form suited for systemic injection or infusion and may, as such, be formulated with sterile water or an isotonic saline or glucose solution. Formulations may further include one or more diluents, fillers, emulsifiers, preservatives, buffers, excipients, and the like, and may be provided in such forms as liquids, powders, emulsions, suppositories, liposomes, transdermal patches and tablets, for example.

Pharmaceutical compositions of the present invention are administered at daily to weekly intervals. An "effective amount" of such a pharmaceutical composition is an amount that provides a clinically significant decrease in a deleterious T cell-mediated immune response to an autoantigen, for example, those associated with IDDM, or provides other pharmacologically beneficial effects. Such amounts will depend, in part, on the particular condition to be treated, age, weight, and general health of the patient, and other factors evident to those skilled in the art. Preferably the amount of the soluble, fused MHC heterodimer:peptide complex administered will be within the range of 20-80 μg/kg. Compounds having significantly enhanced half-lives may be administered at lower doses or less frequently.

Adjuvants

An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bortadella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be used as adjuvants.

The compositions may also include a *Mycobacterium* species CWS adjuvant, as described above. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic peptide.

Certain adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL® adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science 273*:352, 1996. Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins . Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A, β-escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamelar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol^{R} to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL® adjuvant, as described in WO 94/00153, or a less reactogenic composition

where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other formulations comprise an oil-in-water emulsion and tocopherol. Another adjuvant formulation employs QS21, 3D-MPL® adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 as disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Additional illustrative adjuvants for use in the pharmaceutical compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhanzyn®) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula (I): $HO(CH_2CH_2O)_n$-A-R,

wherein, n is 1-50, A is a bond or –C(O)-, R is C_{1-50} alkyl or Phenyl C_{1-50} alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein *n* is between 1 and 50, preferably 4-24, most preferably 9; the *R* component is C_{1-50}, preferably C_4-C_{20} alkyl and most preferably C_{12} alkyl, and *A* is a bond. The concentration of the polyoxyethylene ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-steoryl ether, polyoxyethylene-8-steoryl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl ether are described in the Merck index (12^{th} edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

Liposome-, Nanocapsule-, and Microparticle-Mediated Delivery

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into the subjects. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the compositions disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (*see*, for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon & Papahadjopoulos, 1988; Allen and Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome-like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used effectively to introduce genes, drugs (Heath & Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta & Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller & Baltimore, 1984), transcription factors and allosteric effectors (Nicolau & Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b;

Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori & Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, *i.e.*, in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977, 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution. However, a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for hours or days, depending on their composition, and half lives in the blood range from minutes to several hours. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominant site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential

targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be are easily made, as described (Couvreur *et al.*, 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

The following example is provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

EXAMPLES

Example 1: Construction of DNA sequences encoding human soluble, fused MHC heterodimer:peptide complexes, HLA-DR4 molecules(CO563 and CO564).

DNA constructs encoding gp39 β1/α1 human molecules were prepared from a cDNA encoding the gp39 peptide fused to the β1/α1 domains of HLA-DR4 according to standard techniques. For the production of "empty" β1/α1 DR4 molecules, a cDNA encoding the fused β1/α1 domains of HLA-DR4 was prepared using cloned α and β chains from DR4.

Amino acid sequence of the gp39-β1/α1 HLA-DR4 human single chain molecule (linkers are shown in bold)

MGDTGRSFTLASSETGVG**ASGGGGSGGG**GDTRPRFLEQVKHECH FFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQ KRAAVDTYCRHNYGVGESFTVQRR**GG**IKEEHVIIQAEFYLNPDQSGEFMFDFDG DEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPIT N*

aa1 – aa4: leader sequence

aa5 - aa18: gp39 peptide

aa19 - aa28: linker

aa29 - aa122: HLA-DR4 β1 domain

aa123 – aa124: linker

aa125 – aa 208: HLA-DR4 α1 domain

Amino acid sequence of the "empty" β1/α1 HLA-DR4 human single chain molecule (linkers in bold):

MGDTRPRFLEQVKHECHFFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTEL GRPDAEYWNSQRDLLEQKRAAVDTYCRHNYGVGESFTVQRR**GG**IKEEHVIIQAE FYLNPDQSGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAV DKANLEIMTKRSNYTPITN*

aa 1 – aa95: HLA-DR4 β1 domain

aa96- aa97: linker

aa98- aa181: HLA-DR4 α1 domain

For the production of recombinant proteins, the bacteria (pLysS) were grown in LB (containing ampicillin (50 μg / ml) and chloramphenicol (5 μg / ml)) at 37°C until OD 600 = 0.5. IPTG was added at the final concentration of 0.5 mM final and the bacteria were further incubated for 3 hours at 37°C with shaking. The bacteria were centrifuged at 4°C, 4000xg for 20 min and the pellet was frozen at –80°C. The following day, the pellet was resuspended in 40 ml of lysis buffer (50 mM Tris-HCl pH 8, 50 mM NaCl, 2 mM EDTA, 1 protease inhibitor cocktail tablet, 1% Triton X100 and 1% deoxycholate), and incubated for 1 hour at 4°C under continuous agitation with a magnetic stirrer. The sample was then homogenized using a French Press with a 16,000 psi setting, and centrifuged at 4°C, 9000g for 20 min. The pellet was then resuspended in 30 ml of lysis buffer without Triton and deoxycholate and centrifuged at 4°C, 9000 g for 20 min. The new pellet was resuspended in 10 ml of 20 mM ethanolamine/6 M urea pH 10, and eventually frozen at –80°C. The recombinant protein was then purified by FPLC ion-exchange chromatography using Source 30Q anion-exchange media in an XK26/20 column using a step gradient going from 1 mM to 1 M NaCl in 20 mM ethanolamine/6M urea pH 10. Fractions were analyzed by SDS/PAGE and those corresponding to the proteins of interest are pooled and dialyzed against PBS 1X.

Example 2: Production of additional single chain constructs

Additional constructs with different composition and length of the 2nd linker (between β1 and α1) were engineered by using standard techniques using CO567 as the template. Specifically, PCR primers were designed to replace the old sequence in CO567 with the new sequence. For example, to make CO581, the primers were designed with the following sequences (note these primers were phosphorylated at 5').

Primer 1:

5' pCACCAGGAGGAGAGCCGCCCACGCCGGTCTCGCTGG

Primer 2:

5' pGACCACCTGGATCTGGGGACACCCGACCACGTTTC

PCR reaction (100 μl) was made of the following components: 2 μl CO567 (80 ng) as template, 2 μl each of primer 1 and primer 2 (10 μM), 2 μl of dNTP

mix (20 mM each), 10 µl of 10X pfu buffer, and 80 µl of sterile water. After all the components were mixed, 2 µl of Turbo pfu (5U total) was added, mixed and put on PCR machine. The PCR cycles has a pre-denaturation at 95°C for 30 sec, then 10 cycles of 95°C for 30°C, 60°C for 1 min, and 72°C for 7 min. Then another 22 cycles of 95°C for 30°C, 65°C for 1 min and 72°C for 7 min, followed by a final 10 min at 72°C.

The PCR mixture was digested with 2 µl of DpnI (10U) for 2h at 37°C. Then the PCR product at ~6 kb was purified from agarose gel after electrophoresis. The purified PCR product was ligated by T4 DNA ligase for 1 h at room temperature then used to transform into NovaBlue (Novagen)competent cells by standard protocol. Cells were plated on LB (+Carb) and grow overnight at 37°C.

Next day, about a dozen single colony from the transformation were randomly picked for overnight culture in 5 ml LB (+Carb) at 37°C. Plasmids from these culture were purified with Wizard Miniprep kit, and analyzed by Xho I digestion. A few plasmids that passed the Xho I digestion were further confirmed by DNA sequencing.

To express the recombinant proteins, a clone with confirmed DNA sequence was used to transform BL21(DE3)CodonPlus-RIL (Stratagene) by standard transformation protocol and plated on LB (Carb+Cam) plates overnight at 37°C. Next morning, a single colony was picked to inoculate 100 ml LB (+Carb +Cam) and the culture was grown till OD reach between 0.8-1.0, and stored overnight at 4°C. Next day, the culture was pellet down and used to inoculate into 2xYT (+Carb+Cam) at ratio of 25 ml culture per liter new media. These large cultures were grown at 37°C till OD=0.5-0.6, and IPTG was added to induce recombinant protein at 37°C for 3 h. The induced cultures were pellet and stored at –80°C till purification.

Table 1 provides a listing of various constructs made according to the invention.

TABLE I

Construct	peptide	upstream linker	downstream linker
C0523	yes	GGGG	GG
C0543	none	none	GG
C0563	yes	ASGGGSGGG	GG
C0567	yes	ASGGGSGGG	TSGGGGSGGGGSSS
C0580	yes	ASGGGSGGG	GSPGGGGSGGGPGS
C0581	yes	ASGGGSGGG	GSPPGGPPGS
C0582	yes	ASGGGSGGG	GSPGGGGPGS
C0583	yes	ASGGGSGGG	TSGGGGS
C0584	yes	ASGGGSGGG	SGGSGGS
C0585	yes	ASGGGSGGG	FDAPSPLP
C0586	none	none	TSGGGGSGGGGSSS
C0587	none	none	GSPGGGGSGGGPGS
C0588	none	none	GSPPGGPPGS
C0589	none	none	GSPGGGGPGS
C0590	none	none	TSGGGGS
C0591	none	none	SGGSGGS
C0592	none	none	FDAPSPLP
CO593	yes	ASGGGSGGG	VYPEVTV
CO594	none	none	VYPEVTV
CO595	yes	ASGGGSGGG	GGGG
CO596	yes	ASGGGSGGG	GGGGS
CO597	yes	ASGGGSGGG	GGGSGG

Example 3: Four classes of novel linkers for MHC class II single chain molecules

Inspection of circular dichroism spectra of purified, refolded single chain constructs indicated that novel linkers could be used for the constructs. The atomic structures of the various murine and human MHC class II molecules, as determined by X-ray crystallography, indicated that these molecules have a high degree of structural similarity. The circular dichroism results were consistent with two folded molecules of clearly different secondary structure. Careful inspection of the structures reveals that the human MHC has a longer distance between chains than the equivalent murine molecule. This results led to proposing longer linkers between the chains which would contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). These constructs make up the first class of linkers. To inhibit the continuation of secondary structure across the linker, prolines were added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. These linkers make up the second class of linkers disclosed here.

Next, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. These are the third class of linkers. Finally, a combination of these types of linkers could also be used. These are the fourth class of linkers.

Example 4: Human MHC class II single chain molecule with murine linkers

Another linker has been suggested based on a combination of murine and human MHC class II single chain molecules. This fusion would incorporate linker residues from the functional murine single chain MHC class II molecule and the alpha and beta chains of the human molecule. The protein sequence of the single chain molecule is provided below (the linker residues from the mouse construct are in bold):

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECHFFNGTERVRF
LDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQKRAAVDTYC
RHNYGVGESFTV**LRRLGGEDDEADH**HVIIQAEFYLNPDQSGEFMFDFDGDEIFH
VDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITN*

Further MHC class II hybrid single chain molecules may be designed by fusing other portions of the alpha chain and the beta chain together using linkers as described elsewhere. A properly folded molecule may be obtained by putting appropriate

linkers between portions of the human MHC class II which are proximal to each other as determined by visual inspection of the atomic coordinates of residues of the native MHC available in the publicly accessible protein structure database. These structures would predict possible fusion proteins which covalently attach any part of the beta chain between residues 82 to 123 or between residues 148 to 164 to portions of the alpha chain such as the N-terminal residues, residues 79 to 84, or 92 to 106. The numbering system of residues in this example corresponds to those found in the coordinates of the structure described in: DESSEN, *et al. Immunity* 7:473 (1997). Other, homologous residues could be used to create equivalent constructs for genotypic and allelic variants of these molecules e.g. equivalent residues in DR2 or such. DNAs for such hybrids would be prepared and expressed in a recombinant expression system by someone skilled in the art and could be assayed for structure and function in appropriate assays.

Example 5: Use of CD4 binding site(s) of MHC class II molecules as linkers for the production of bioactive recombinant MHC class II:peptide complexes

HLA class II molecules present antigenic peptides to the T cell receptor of the CD4+ T lymphocytes and interact with CD4 during the antigen recognition process. Native MHC class II-peptide complexes have been shown to bind to MHC class II restricted and antigen specific TCRs on a particular T cell and induce T cell nonresponsiveness. It is proposed that the CD4 binding site is important in the docking of MHC class II-peptide complex with the TCR and induce nonresponsiveness. Since the binding of CD4 to MHC class II-peptide is important in antigen presentation and/or induction of T cell nonresponsiveness, it is proposed that recombinant MHC class II-peptide molecules (truncated or whole) containing CD4 binding site will be biologically active. Furthermore, a polypeptide fragment from MHC class II which binds the CD4, when used as a linker in preparation of MHC class II-peptide truncated molecules, provides resulting recombinant molecules that will be biologically active.

The following describes the concept of different linkers. Peptide is attached to a linker 1 (L1) which is attached to N-terminus of β1 domain that is linked to L2. L2 is linked to L3, which in turn is linked to N-terminus of α1 chain of MHC class II. Here L2 represents the human CD4 binding sequences. It should be noted that L2 could also be directly linked to N-terminus of α1 domain by completely deleting L3.

Specific examples of L1 and L3 are given in a examples. The sequences of L2 are given below. These sequences are applicable to most of the DR-Peptide molecules. RNGQEEKAGVVSTGLI, RNGQETKAGVVSTGLI, YNQQEEKAGGVSTGLI, FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI, FYNQQEEKAGGVSTGLI, and LNGQEEKAGMVSTGLI.

Example 6: I-As MBP.β1β2α1α2.Cκ construct and activity

The "full anergix" single chain molecule, mouse I-As MBP.β1β2α1α2.Cκ, was generated by overlap PCR using standard methodology. The molecules was expressed in 293T cells and baculoviral cells according to standard methodology, and purified according to standard methodology using affinity chromatography using goat-anti mouse antibodies. The structure of I-As MBP.β1β2α1α2.Cκ is shown in Figure 1, and a comparison of its structure to a native murine molecule is shown in Figure 2. Figure 3 shows silver staining and western analysis of the recombinant proteins.

The amino acid sequence of the I-As MBP.β1β2α1α2.Cκ shown in Figure 1 is as follows:

METDTLLLWVLLLWVPGSTGDFKNIVTPRTPPPASGGGGSGGGGDSERHFVFQF
KGECYFTNGTQRIRSVDRYIYNREEYLRFDSDVGEYRAVTELGRPDPEYYNKQY
LEQTRAELDTVCRHNYEGVETHTSLRRLEQPNVVISLSRTEALNHHNTLVCSVTD
FYPAKIKVRWFRNGQEETVGVSSTQLIRNGDWTFQVLVMLEMTPRRGEVYTCH
VEHPSLKSPITVEWTSGGGGSGGGGSGGGGSGGGGSSSEDDIEADHVGVYGTTV
YQSPGDIGQYTHEFDGDEWFYVDLDKKETIWMLPEFGQLTSFDPQGGLQNIATG
KYTLGILTKRSNSTPATNEAPQATVFPKSPVLLGQPNTLICFVDNIFPPVINITWLR
NSKSVTDGVYETSFLVNRDHSFHKLSYLTFIPSDDDIYDCKVEHWGLEEPVLKHW
ASGGGGSGGGGADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDG
SERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVK
SFNRNEC

The *in vitro* activity of recombinant I-As MBP.β1β2α1α2.Cκ was tested using MBP90-101 specific IAs restricted mouse T cell clone HS1. This clone was prepared by the immunization of SJL mice with the MBP90-101 peptide, followed by cloning out CD4+ T cells by limited dilution techniques. These cells were maintained by stimulation every 10 days with irradiated SJF splenocytes and PBP90-101 peptide. The T

cells are activated by a combination of soluble recombinant I-As MBP.β1β2α1α2.Cκ and plate bound anti-CD28 antibody (see Figure 4). T cell activation was assayed by ^{3}H-thymidine incorporation according to standard methodology. Figure 5 shows the results of this assay.

MS is a T cell dependent autoimmune disease caused by localized demyelination in the central nervous system. Experimental autoimmune encephalomyelitis is a accepted animal model of MS. The following results demonstrate that administration of I-As MBP.β1β2α1α2.Cκ reduces the incidence and severity of EAE. EAE was induced according to standard methodology according to the myelin model. Ten μg of recombinant I-As MBP.β1β2α1α2.Cκ was given intravenously in 100 μl of PBS at days 1, 4, 7, and 11 after disease induction. 12 days after immunization, animals are observed daily for the onset of neurological dysfunction. Disease is graded by trained technicians according to standard methods (see Figure 6). Mice are followed for up to 60-70 days. The date shown in Figure 7 demonstrate that administration of the recombinant I-As MBP.β1β2α1α2.Cκ significantly reduced the incidence of myelin-induced EAE in SJL mice. In one experiment, 11 out of 20 mice developed EAE in the untreated group (55%), whereas only 2 out of 21 animal showed sign of the disease in the treated group (16.6%). Similarly, in another experiment, 12 out of 16 mice developed EAE in the untreated group (75%), while only 2 out of 16 developed EAE in the treated group (12.5%). Figure 8 shows histological data.

Example 7: Functional human anergix molecules optimized for *E. coli* expression with *E. coli* codons

Two human single chain MHC class II heterodimer molecules (CO528-AC and CO608-AC) have been optimized for *E. coli* expression using "artificial codons," e.g., preferred *E. coli* codons encoding the mammalian protein. CO528-AC and CO608-AC were made according to standard PCR overlap technology.

Example 7: Recombinant MHC class II IAs.MBP.Ig multimeric complexes

Recombinant MHC class II (IAs)-peptide–Ig fusion complexes were constructed by fusing the mIgG leader, MBP 90-101, or MBP1-14 (as a control) to IAs single chain (b1b2a1a2), and mIgG.Ck, mIgG.CH1.H, mIgG.CH1.H.CH2, or

mIgG.CH1.H.CH2.CH3 with flexible linkers, according to standard methodology. The recombinant IAs fusion proteins were expressed in both mammalian and insect cells and detected by western analysis and ELISA. The overexpressed and secreted recombinant IAs fusion proteins from both human 293 cell cultures or from insect culture medium were purified by affinity chromatography. The purified dimeric and tetrameric recombinant IAs proteins have *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant IAs fusion proteins were studied with the experimental autoimmune encephalomyelitis (EAE) model using susceptible SJL mice. In these EAE studies, recombinant IAs fusion protein was delivered on days 1, 4, 7, and 11 by I.V. injections after induction of the disease with myelin. The animals were then examined for neurological dysfunction. The results indicate that treatment with the recombinant IAs fusion proteins prevents mortality and significantly reduces paralysis induced by myelin homogenate in CFA. In conclusion, these studies suggest that the recombinant MHC class II fusion protein has therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

Example 8: Synthesis of mouse model equivalent of CO608

Four forms of the murine MHC Class II IAs β1α1 with MBP peptide linkers analogous to CO608 human were made. These constructs can be used, e.g., as murine clinical control for human 608. mCO608 (mouse CO608), MCO608-A (lacking first four amino acids from beta 1 domain), *B. megaterium*-mCO608-A (expressed in *Bacillus megaterium*), and mCO608-B (first four amino acids replaced).

To make mCO608, the upstream linker of mouse CO521 (GGGS) was replaced with the human CO608 linker (ASGGGGSGGG) and the downstream linker of mouse CO521 (GG) was replaced with the downstream linker of CO608 (TSGGGGS), using PCR according to standard methodology. mCO608A was made from mCO608 using PCR according to standard methodology.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
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Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
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Gly Gly Ser Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu
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Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp
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Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp
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Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
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Gly Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr
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Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu
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35 40 45

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Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
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Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser
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Gly Gly Ser Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala
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Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe
115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val
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Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly
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Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
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Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
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Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
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Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr
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Ser Gly Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala
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Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe
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Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val
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Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
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Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
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Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Ser Pro Gly Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile
100 105 110

Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met
115 120 125

Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys
130 135 140

Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu
145 150 155 160

Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu
165 170 175

Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> CO588
<211> 190
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser

50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Ser Pro Pro Gly Gly Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile
100 105 110

Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met
115 120 125

Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys
130 135 140

Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu
145 150 155 160

Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu
165 170 175

Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
180 185

<210> CO587
<211> 194
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly
85 90 95

Ser Pro Gly Gly Gly Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu
100 105 110

Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser
115 120 125

Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp
130 135 140

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe
145 150 155 160

Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys
165 170 175

Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr
180 185 190

Asn

<210> CO586
<211> 194
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr
85 90 95

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu
100 105 110

Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser
115 120 125

Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp
130 135 140

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe
145 150 155 160

Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys
165 170 175

Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr
180 185 190

Asn

<210> CO598

<211> 639
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
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gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgctg 360
cgccgactcg gaggtgaaga tgacgaggca gatcaccatg tgatcatcca ggccgagttc 420
tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatggtga tgagatttc 480
catgtggata tggcaaagaa ggagacggtc tggcggcttg aagaatttgg acgatttgcc 540
agctttgagg ctcaaggtgc attggccaac atagctgtgg acaaagccaa cctggaaatc 600
atgacaaagc gctccaacta tactccgatc accaattaa 639

<210> CO597
<211> 639
<212> DNA
<213> Homo sapiens

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
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gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggag gtggaagcgg cggaatcaaa gaagaacatg tgatcatcca ggccgagttc 420
tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatggtga tgagatttc 480
catgtggata tggcaaagaa ggagacggtc tggcggcttg aagaatttgg acgatttgcc 540
agctttgagg ctcaaggtgc attggccaac atagctgtgg acaaagccaa cctggaaatc 600
atgacaaagc gctccaacta tactccgatc accaattaa 639

<210> CO596
<211> 636
<212> DNA
<213> Homo sapiens

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ggaggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggag gtggaggcag catcaaagaa gaacatgtga tcatccaggc cgagttctat 420
ctgaatcctg accaatcagg cgagtttatg tttgactttg atggtgatga gattttccat 480
gtggatatgg caaagaagga gacggtctgg cggcttgaag aatttggacg atttgccagc 540
tttgaggctc aaggtgcatt ggccaacata gctgtggaca aagccaacct ggaaatcatg 600
acaaagcgct ccaactatac tccgatcacc aattaa 636

<210> CO595
<211> 633
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240

ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggag gtggaggcat caaagaagaa catgtgatca tccaggccga gttctatctg 420
aatcctgacc aatcaggcga gtttatgttt gactttgatg gtgatgagat tttccatgtg 480
gatatggcaa agaaggagac ggtctggcgg cttgaagaat ttggacgatt tgccagcttt 540
gaggctcaag gtgcattggc caacatagct gtggacaaag ccaacctgga aatcatgaca 600
aagcgctcca actatactcc gatcaccaat taa 633

<210> CO593
<211> 642
<212> DNA
<213> Homo sapiens

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ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgagtct accctgaggt aactgtcatc aaagaagaac atgtgatcat ccaggccgag 420
ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 480
ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540
gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600
atcatgacaa agcgctccaa ctatactccg atcaccaatt aa 642

<210> CO585
<211> 645
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgattcg acgcacctag cccactccca atcaaagaag aacatgtgat catccaggcc 420
gagttctatc tgaatcctga ccaatcaggc gagtttatgt ttgactttga tggtgatgag 480
attttccatg tggatatggc aaagaaggag acggtctggc ggcttgaaga atttggacga 540
tttgccagct ttgaggctca aggtgcattg gccaacatag ctgtggacaa agccaacctg 600
gaaatcatga caaagcgctc caactatact ccgatcacca attaa 645

<210> CO584
<211> 642
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaagtg gcggtagtgg cggtagtatc aaagaagaac atgtgatcat ccaggccgag 420
ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 480
ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540
gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600
atcatgacaa agcgctccaa ctatactccg atcaccaatt aa 642

<210> CO583
<211> 642
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaacta gtggtggcgg tggcagcatc aaagaagaac atgtgatcat ccaggccgag 420
ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 480
ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540
gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600
atcatgacaa agcgctccaa ctatactccg atcaccaatt aa 642

<210> CO582
<211> 651
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggct ctcctggagg tggaggtcct ggatctatca aagaagaaca tgtgatcatc 420
caggccgagt tctatctgaa tcctgaccaa tcaggcgagt ttatgtttga ctttgatggt 480
gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt 540
ggacgatttg ccagctttga ggctcaaggt gcattggcca acatagctgt ggacaaagcc 600
aacctggaaa tcatgacaaa gcgctccaac tatactccga tcaccaatta a 651

<210> CO581
<211> 651
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggct ctcctcctgg tggaccacct ggatctatca aagaagaaca tgtgatcatc 420
caggccgagt tctatctgaa tcctgaccaa tcaggcgagt ttatgtttga ctttgatggt 480
gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt 540
ggacgatttg ccagctttga ggctcaaggt gcattggcca acatagctgt ggacaaagcc 600
aacctggaaa tcatgacaaa gcgctccaac tatactccga tcaccaatta a 651

<210> CO580
<211> 663
<212> DNA
<213> Homo sapiens

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atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaggga gtccaggcgg tggcgggagc ggcggtggtc ctgggagtat caaagaagaa 420
catgtgatca tccaggccga gttctatctg aatcctgacc aatcaggcga gtttatgttt 480
gactttgatg gtgatgagat tttccatgtg gatatggcaa agaaggagac ggtctggcgg 540
cttgaagaat ttggacgatt tgccagcttt gaggctcaag gtgcattggc caacatagct 600
gtggacaaag ccaacctgga aatcatgaca aagcgctcca actatactcc gatcaccaat 660
taa                                                               663
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<210> CO567
<211> 663
<212> DNA
<213> Homo sapiens

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atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggcgctagc 60
ggaggggggcg gaagcggcgg agggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360
cggcgaacta gtggtggcgg tggcagcggc ggtggtggtt cctcgagtat caaagaagaa 420
catgtgatca tccaggccga gttctatctg aatcctgacc aatcaggcga gtttatgttt 480
gactttgatg gtgatgagat tttccatgtg gatatggcaa agaaggagac ggtctggcgg 540
cttgaagaat ttggacgatt tgccagcttt gaggctcaag gtgcattggc caacatagct 600
gtggacaaag ccaacctgga aatcatgaca aagcgctcca actatactcc gatcaccaat 660
taa                                                               663
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<210> CO598
<211> 213
<212> PRT
<213> Homo sapiens

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Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
                5                  10                  15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
            20                  25                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
        35                  40                  45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
    50                  55                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65                  70                  75                  80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
                85                  90                  95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
            100                 105                 110
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Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu Gly Gly Glu Asp Asp
115 120 125

Glu Ala Asp His His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro
130 135 140

Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe
145 150 155 160

His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe
165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala
180 185 190

Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr
195 200 205

Pro Ile Thr Asn
210

<210> CO597
<211> 213
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Ser Gly Gly
115 120 125

Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro
130 135 140

Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe
145 150 155 160

His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe
165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala
180 185 190

Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr
195 200 205

Pro Ile Thr Asn
210

<210> CO596
<211> 212
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Gly Ser Ile
115 120 125

Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp
130 135 140

Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His
145 150 155 160

Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly
165 170 175

Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val
180 185 190

Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro
195 200 205

Ile Thr Asn
210

<210> CO595
<211> 211
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Gly Ile Lys
115 120 125

Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln
130 135 140

Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val
145 150 155 160

Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg
165 170 175

Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp
180 185 190

Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile
195 200 205

Thr Asn
210

<210> CO593
<211> 214
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val Tyr Pro Glu Val Thr
115 120 125

Val Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn
130 135 140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile
145 150 155 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu
165 170 175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile
180 185 190

Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr
195 200 205

Thr Pro Ile Thr Asn
210

<210> CO585
<211> 215
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe Asp Ala Pro Ser Pro
115 120 125

Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu
130 135 140

Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu
145 150 155 160

Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu
165 170 175

Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn
180 185 190

Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn
195 200 205

Tyr Thr Pro Ile Thr Asn
210

<210> CO584
<211> 214
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser Gly Gly Ser Gly Gly
115 120 125

Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn
130 135 140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile

145 150 155 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu
165 170 175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile
180 185 190

Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr
195 200 205

Thr Pro Ile Thr Asn
210

<210> CO583
<211> 214
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly
115 120 125

Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn
130 135 140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile
145 150 155 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu
165 170 175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile
180 185 190

Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr
195 200 205

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Thr Pro Ile Thr Asn
    210
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<210> CO582
<211> 217
<212> PRT
<213> Homo sapiens

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Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
                  5                  10                  15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
             20                  25                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
         35                  40                  45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
     50                  55                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
 65                  70                  75                  80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
                 85                  90                  95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
            100                 105                 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly
        115                 120                 125

Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe
    130                 135                 140

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly
145                 150                 155                 160

Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg
                165                 170                 175

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu
            180                 185                 190

Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg
        195                 200                 205

Ser Asn Tyr Thr Pro Ile Thr Asn
    210                 215
```

<210> CO581
<211> 217
<212> PRT
<213> Homo sapiens

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Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
                  5                  10                  15
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```
Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
            20                  25                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
        35                  40                  45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
    50                  55                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65                  70                  75                  80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
                85                  90                  95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
            100                 105                 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Pro Gly Gly
        115                 120                 125

Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe
    130                 135                 140

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly
145                 150                 155                 160

Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg
                165                 170                 175

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu
            180                 185                 190

Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg
        195                 200                 205

Ser Asn Tyr Thr Pro Ile Thr Asn
    210                 215
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<210> CO580
<211> 221
<212> PRT
<213> Homo sapiens

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Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
                5                   10                  15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
            20                  25                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
        35                  40                  45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
    50                  55                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
```

65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly
115 120 125

Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile
130 135 140

Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe
145 150 155 160

Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu
165 170 175

Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala
180 185 190

Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile
195 200 205

Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
210 215 220

<210> CO567
<211> 221
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly
115 120 125

```
Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu Glu His Val Ile Ile
    130                 135                 140

Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe
145                 150                 155                 160

Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu
                165                 170                 175

Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala
            180                 185                 190

Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile
        195                 200                 205

Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn
    210                 215                 220
```

I-As MBP.β1β2α1α2.Cκ nucleotide sequence

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gcggccgcca ccatggagac agacacactc ctgctatggg tactgctgct ctgggttcca 60
ggttccactg gtgacttcaa gaacattgtg acacctcgaa caccacctcc agctagcgga 120
gggggcggaa gcggcggagg gggagactcc gaaaggcatt tcgtgttcca gttcaagggc 180
gagtgctact tcaccaacgg gacgcagcgc atacgatctg tggacagata catctacaac 240
cgggaggagt acctgcgctt cgacagcgac gtgggcgagt accgcgcggt gaccgagctg 300
gggcggccag accccgagta ctacaataag cagtacctgg agcaaacgcg ggccgagctg 360
gacacggtgt gcagacacaa ctacgagggg gtggagaccc acacctccct gcggcggctt 420
gaacagccca atgtcgtcat ctccctgtcc aggacagagg ccctcaacca ccacaacact 480
ctggtctgct cagtgacaga tttctaccca gccaagatca aagtgcgctg gttccggaat 540
ggccaggagg agacggtggg ggtctcatcc acacagctta ttaggaatgg ggactggacc 600
ttccaggtcc tggtcatgct ggagatgacc cctcggcggg gagaggtcta cacctgccac 660
gtggagcatc cgagcctgaa gagccccatc actgtggagt ggactagtgg tggcggtggc 720
agcggcggtg gtggttccgg tggcggcggt tctggcggtg gcggttcctc gagtgaagac 780
gacattgagg ccgaccacgt aggcgtctat ggtacaactg tatatcagtc tcctggagac 840
attggccagt acacacatga atttgatggt gatgagtggt tctatgtgga cttggataag 900
aaggagacta tctggatgct tcctgagttt ggccaattga caagctttga cccccaaggt 960
ggactgcaaa acatagctac aggaaaatac accttgggaa tcttgactaa gaggtcaaat 1020
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ctgggtcagc ccaacaccct catctgcttt gtggacaaca tcttccctcc tgtgatcaac 1140
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gtcaaccgtg accattcctt ccacaagctg tcttatctca ccttcatccc ttctgacgat 1260
gatatttatg actgcaaggt ggagcactgg ggcctggagg agccggttct gaaacactgg 1320
gctagcggag ggggcggaag cggcggaggg ggagctgatg ctgcaccaac tgtatccatc 1380
ttcccaccat ccagtgagca gttaacatct ggaggtgcct cagtcgtgtg cttcttgaac 1440
aacttctacc ccaaagacat caatgtcaag tggaagattg atggcagtga acgacaaaat 1500
ggcgtcctga acagttggac tgatcaggac agcaaagaca gcacctacag catgagcagc 1560
accctcacgt tgaccaagga cgagtatgaa cgacataaca gctatacctg tgaggccact 1620
cacaagacat caacttcacc cattgtcaag agcttcaaca ggaatgagtg ttagggtacc 1680
```

I-As MBP.β1β2α1α2.Cκ amino acid sequence

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
                5                  10                  15

Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro
            20                  25                  30
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Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg
35 40 45

His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr
50 55 60

Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr
65 70 75 80

Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu
85 90 95

Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr
100 105 110

Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly Val Glu
115 120 125

Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val Ile Ser
130 135 140

Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val Cys Ser
145 150 155 160

Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe Arg Asn
165 170 175

Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile Arg Asn
180 185 190

Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr Pro Arg
195 200 205

Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu Lys Ser
210 215 220

Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
225 230 235 240

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Glu Asp
245 250 255

Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln
260 265 270

Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu
275 280 285

Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro
290 295 300

Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn
305 310 315 320

Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn
325 330 335

Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe Pro Lys

340 345 350

Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe Val Asp
355 360 365

Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys
370 375 380

Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp
385 390 395 400

His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp
405 410 415

Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val
420 425 430

Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala
435 440 445

Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu
450 455 460

Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro
465 470 475 480

Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn
485 490 495

Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr
500 505 510

Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His
515 520 525

Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile
530 535 540

Val Lys Ser Phe Asn Arg Asn Glu Cys
545 550

<210> CO528-AC
<211> 600
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggtggtggc 60
ggttcgcgtc cacgtttctt ggaacaggtt aaacatgagt gtcatttttt caatgggacg 120
gaacgcgtgc gttttctgga tcgttacttt tatcaccaag aggaatacgt acgcttcgac 180
agcgatgtgg gcgaatatcg tgcggtcacg gaactgggtc gtcctgatgc cgaatactgg 240
aacagtcaga aggacttact ggagcagaaa cgtgcagcgg tggataccta ttgccgccac 300
aattacggcg ttggtgaaag cttcacagtc cagcgtcgcg gtggcatcaa agaagagcat 360
gtgattatcc aggcggaatt ctatctgaat ccggatcaat cgggcgaatt catgtttgac 420
ttcgatggtg atgagatttt ccatgttgat atggcaaaga aagaaacggt ctggcgctta 480
gaggaatttg gccgctttgc ctcgttcgaa gctcaaggcg cattggccaa cattgctgtg 540
gataaagcga acctggaaat catgacaaaa cgctccaact atactccgat taccaattaa 600

<210> CO608-AC
<211> 642
<212> DNA
<213> Homo sapiens

atgggggaca ccggtcgcag cttcaccctg gcctccagcg agaccggcgt gggtgcttct 60
ggcgggggcg gttcgggcgg tgggggtgac acccgtccac gtttcttgga acaggttaaa 120
catgagtgtc atttttcaa tgggacggaa cgcgtgcgtt ttctggatcg ttacttttat 180
caccaagagg aatacgtacg cttcgacagc gatgtgggcg aatatcgtgc ggtcacggaa 240
ctgggtcgtc ctgatgccga atactggaac agtcagaagg acttactgga gcagaaacgt 300
gcagcggtgg atacctattg ccgccacaat tacggcgttg gtgaaagctt cacagtccag 360
cgtcgcacta gtggtggcgg tggctctatc aaagaagagc atgtgattat ccaggcggaa 420
ttctatctga atccggatca atcgggcgaa ttcatgtttg acttcgatgg tgatgagatt 480
ttccatgttg atatggcaaa gaaagaaacg gtctggcgct tagaggaatt tggccgcttt 540
gcctcgttcg aagctcaagg cgcattggcc aacattgctg tggataaagc gaacctggaa 600
atcatgacaa aacgctccaa ctatactccg attaccaatt aa 642

<210> CO528
<211> 600
<212> DNA
<213> Homo sapiens

atgggggaca ccggaagatc gttcacactc gcatcatcag agacaggagt aggaggagga 60
ggatcgcgac cacgtttctt ggagcaggtt aaacatgagt gtcatttctt caacgggacg 120
gagcgggtgc ggttcctgga cagatacttc tatcaccaag aggagtacgt gcgcttcgac 180
agcgacgtgg gggagtaccg ggcggtgacg gagctggggc ggcctgatgc cgagtactgg 240
aacagccaga gggacctcct ggagcagaag cgggccgcgg tggacaccta ctgcagacac 300
aactacgggg ttggtgagag cttcacagtg cagcggcgag gaggtatcaa agaagaacat 360
gtgatcatcc aggccgagtt ctatctgaat cctgaccaat caggcgagtt tatgtttgac 420
tttgatggtg atgagatttt ccatgtggat atggcaaaga aggagacggt ctggcggctt 480
gaagaatttg gacgatttgc cagctttgag gctcaaggtg cattggccaa catagctgtg 540
gacaaagcca acctggaaat catgacaaag cgctccaact atactccgat caccaattaa 600

<210> CO608
<211> 630
<212> DNA
<213> Homo sapiens

ATGGGGGACACCCGCAGCTTCACCCTGGCCTCCAGCGAGACCGGCGTGGGCGCTAGCGGAGGGGGCGGAAGC
GGCGGAGGGGGGCCACGTTTCTTGGAGCAGGTTAAACATGAGTGTCATTTCTTCAACGGGACGGAGCGGGTG
CGGTTCCTGGACAGATACTTCTATCACCAAGAGGAGTACGTGCGCTTCGACAGCGACGTGGGGGAGTACCGG
GCGGTGACGGAGCTGGGGCGGCCTGATGCCGAGTACTGGAACAGCCAGAAGGACCTCCTGGAGCAGAAGCGG
GCCGCGGTGGACACCTACTGCAGACACAACTACGGGGTTGGTGAGAGCTTCACAGTGCAGCGGCGAACTAGT
GGTGGCGGTGGCAGCATCAAAGAAGAACATGTGATCATCCAGGCCGAGTTCTATCTGAATCCTGACCAATCA
GGCGAGTTTATGTTTGACTTTGATGGTGATGAGATTTTCCATGTGGATATGGCAAAGAAGGAGACGGTCTGG
CGGCTTGAAGAATTTGGACGATTTGCCAGCTTTGAGGCTCAAGGTGCATTGGCCAACATAGCTGTGGACAAA
GCCAACCTGGAAATCATGACAAAGCGCTCCAACTATACTCCGATCACCAATTAA

<210> CO 608 variation
<211> 642
<212> DNA--CO608
<213> Homo sapiens

ATGGGGGACACCGGTCGCAGCTTCACCCTGGCCTCCAGCGAGACCGGCGTGGGCGCTAGCGGAGGGGGCGGA
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ACGGAGCGGGTGCGGTTCCTGGACAGATACTTCTATCACCAAGAGGAGTACGTGCGCTTCGACAGCGACGTG
GGGGAGTACCGGGCGGTGACGGAGCTGGGGCGGCCTGATGCCGAGTACTGGAACAGCCAGAAGGACCTCCTG
GAGCAGAAGCGGGCCGCGGTGGACACCTACTGCAGACACAACTACGGGGTTGGTGAGAGCTTCACAGTGCAG
CGGCGAACTAGTGGTGGCGGTGGCAGCATCAAAGAAGAACATGTGATCATCCAGGCCGAGTTCTATCTGAAT
CCTGACCAATCAGGCGAGTTTATGTTTGACTTTGATGGTGATGAGATTTTCCATGTGGATATGGCAAAGAAG
GAGACGGTCTGGCGGCTTGAAGAATTTGGACGATTTGCCAGCTTTGAGGCTCAAGGTGCATTGGCCAACATA
GCTGTGGACAAAGCCAACCTGGAAATCATGACAAAGCGCTCCAACTATACTCCGATCACCAATTAA

<210> CO528-AC
<211> 200
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
1 5 10 15

Val Gly Gly Gly Gly Ser Arg Pro Arg Phe Leu Glu Gln Val Lys His
20 25 30

Glu Cys His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg
35 40 45

Tyr Phe Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly
50 55 60

Glu Tyr Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp
65 70 75 80

Asn Ser Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr
85 90 95

Tyr Cys Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg
100 105 110

Arg Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr
115 120 125

Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp
130 135 140

Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu
145 150 155 160

Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala
165 170 175

Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser
180 185 190

Asn Tyr Thr Pro Ile Thr Asn
195

<210> CO608-AC
<211> 214
<212> PRT
<213> Homo sapiens

```
Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
                  5                  10                  15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg
             20                  25                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly
         35                  40                  45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu
     50                  55                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu
 65                  70                  75                  80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu
                 85                  90                  95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly
            100                 105                 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly
        115                 120                 125

Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn
    130                 135                 140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile
145                 150                 155                 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu
                165                 170                 175

Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile
            180                 185                 190

Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr
        195                 200                 205

Thr Pro Ile Thr Asn
    210
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<210> CO608
<211> 209
<212> PRT
<213> Homo sapiens

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AVTELGRPDAEYWNSQKDLLEQKRAAVDTYCRHNYGVGESFTVQRRTSGGGGSIKEEHVIIQAEFYLNPDQS
GEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITN

<210> CO608-variation
<211> 211
<212> PRT
<213> Homo sapiens

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECHFFNGTERVRFLDRYFYHQEEYVRFDSDV
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PDQSGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIADKANLEIMTKRSNYTPIT

<210> CO528
<211> 200
<212> PRT
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly
5 10 15

Val Gly Gly Gly Gly Ser Arg Pro Arg Phe Leu Glu Gln Val Lys His
20 25 30

Glu Cys His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg
35 40 45

Tyr Phe Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly
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Glu Tyr Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp
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Asn Ser Gln Arg Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr
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Tyr Cys Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg
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Arg Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr
115 120 125

Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp
130 135 140

Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu
145 150 155 160

Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala
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Asn Tyr Thr Pro Ile Thr Asn
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<210> IAS MBP1-14 CH1.CH2.CH3
<211> 2346
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<210> IAS MBP 1-14 CH1.H
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<210> IAS MBP 90-101 CH1.H.CH2
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<213> murine

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<210> IAS MBP 90-101 CH1.H

<211> 1707
<212> DNA
<213> murine

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<210> MBP 1-14 CK
<211> 1686
<212> DNA
<213> murine

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<210> MBP 1-14 CH1.H.CH2
<211> 2059
<212> DNA
<213> murine

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<211> 2343
<212> DNA--
<213> murine

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gtcaaccgtg accattcctt ccacaagctg tcttatctca ccttcatccc ttctgacgat 1260
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gctagcggag ggggcggaag cggcggagga agcttagcca aaacgacacc cccatctgtc 1380
tatccactgg cccctggatc tgctgcccaa actaactcca tggtgaccct gggatgcctg 1440
gtcaagggct atttccctga gccagtgaca gtgacctgga actctggatc cctgtccagc 1500
ggtgtgcaca ccttcccagc tgtcctgcag tctgacctct acactctgag cagctcagtg 1560
actgtcccct ccagcacctg gcccagcgag accgtcacct gcaacgttgc ccacccggcc 1620
agcagcacca aggtggacaa gaaaattgtg cccagggatt gtggttgtaa gccttgcata 1680
tgtacagtcc cagaagtatc atctgtcttc atcttccccc caaagcccaa ggatgtgctc 1740
accattactc tgactcctaa ggtcacgtgt gttgtggtag acatcagcaa ggatgatccc 1800
gaggtccagt tcagctggtt tgtagatgat gtggaggtgc acacagctca gacgcaaccc 1860
cgggaggagc agttcaacag cactttccgc tcagtcagtg aacttcccat catgcaccag 1920
gactggctca atggcaagga gttcaaatgc agggtcaaca gtgcagcttt ccctgccccc 1980
atcgagaaaa ccatctccaa aaccaaaggc agaccgaagg ctccacaggt gtacaccatt 2040
ccacctccca aggagcagat ggccaaggat aaagtcagtc tgacctgcat gataacagac 2100
ttcttccctg aagacattac tgtggagtgg cagtggaatg ggcagccagc ggagaactac 2160
aagaacactc agcccatcat ggacacagat ggctcttact tcgtctacag caagctcaat 2220
gtgcagaaga gcaactggga ggcaggaaat actttcacct gctctgtgtt acatgagggc 2280
ctgcacaacc accatactga gaagagcctc tcccactctc ctggtaaatg atctggtacc 2340
tgc                                                               2343
```

<210> IASMBP 1-14 CH1.CH2.CH3
<211> 679
<212> PRT--
<213> murine

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
                  5                  10                  15

Gly Ser Thr Gly Asp Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His
             20                  25                  30

Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser
         35                  40                  45

Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn
     50                  55                  60
```

```
Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu
 65                  70                  75                  80

Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr
                85                  90                  95

Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu
            100                 105                 110

Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly
        115                 120                 125

Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val
    130                 135                 140

Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val
145                 150                 155                 160

Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe
                165                 170                 175

Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile
            180                 185                 190

Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr
        195                 200                 205

Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu
    210                 215                 220

Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser
                245                 250                 255

Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val
            260                 265                 270

Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly
        275                 280                 285

Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met
    290                 295                 300

Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu
305                 310                 315                 320

Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg
                325                 330                 335

Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe
            340                 345                 350

Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe
        355                 360                 365

Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn
    370                 375                 380
```

```
Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn
385                 390                 395                 400

Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser
                405                 410                 415

Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu
            420                 425                 430

Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly
        435                 440                 445

Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys
    450                 455                 460

Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly
465                 470                 475                 480

Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser
                485                 490                 495

Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln Ser Gly Leu Tyr Thr
            500                 505                 510

Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr
        515                 520                 525

Val Thr Cys Ser Val Ala His Pro Ala Ser Ser Thr Thr Val Asp Lys
    530                 535                 540

Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile Asn Pro Cys Pro Pro
545                 550                 555                 560

Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly Pro
                565                 570                 575

Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp Val Leu Met Ile Ser
            580                 585                 590

Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp
        595                 600                 605

Pro Asp Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr
    610                 615                 620

Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Ile Arg Val
625                 630                 635                 640

Val Ser Thr Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu
                645                 650                 655

Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ser Pro Ile Glu Arg
            660                 665                 670

Thr Ile Ser Lys Ile Lys
        675
```

<210> IAS MBP 1-14 CH1.H
<211> 677
<212> PRT--
<213> murine

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
5 10 15

Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro
20 25 30

Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg
35 40 45

His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr
50 55 60

Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr
65 70 75 80

Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu
85 90 95

Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr
100 105 110

Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly Val Glu
115 120 125

Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val Ile Ser
130 135 140

Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val Cys Ser
145 150 155 160

Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe Arg Asn
165 170 175

Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile Arg Asn
180 185 190

Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr Pro Arg
195 200 205

Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu Lys Ser
210 215 220

Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
225 230 235 240

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Glu Asp
245 250 255

Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln
260 265 270

Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu
275 280 285

```
Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro
    290                 295                 300

Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn
305                 310                 315                 320

Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn
                325                 330                 335

Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe Pro Lys
            340                 345                 350

Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe Val Asp
        355                 360                 365

Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys
    370                 375                 380

Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp
385                 390                 395                 400

His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp
                405                 410                 415

Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val
            420                 425                 430

Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala
        435                 440                 445

Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys Gly Asp
    450                 455                 460

Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe
465                 470                 475                 480

Pro Glu Ser Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Ser
                485                 490                 495

Val His Thr Phe Pro Ala Leu Leu Gln Ser Gly Leu Tyr Thr Met Ser
            500                 505                 510

Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val Thr
        515                 520                 525

Cys Ser Val Ala His Pro Ala Ser Ser Thr Thr Val Asp Lys Lys Leu
    530                 535                 540

Glu Pro Ser Gly Pro Ile Ser Thr Ile Asn Pro Cys Pro Pro Cys Lys
545                 550                 555                 560

Glu Cys His Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly Pro Ser Val
                565                 570                 575

Phe Ile Phe Pro Pro Asn Ile Lys Asp Val Leu Met Ile Ser Leu Thr
            580                 585                 590

Pro Lys Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp
        595                 600                 605
```

```
Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln
    610                 615                 620

Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Ile Arg Val Val Ser
625                 630                 635                 640

Thr Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys
                645                 650                 655

Cys Lys Val Asn Asn Lys Asp Leu Pro Ser Pro Ile Glu Arg Thr Ile
            660                 665                 670

Ser Lys Ile Lys
        675
```

<210> IAS MBP 90-101 CH1.H.CH2
<211> 563
<212> PRT--
<213> murine

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
                  5                  10                  15

Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro
            20                  25                  30

Pro Ala Ser Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser
        35                  40                  45

Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn
    50                  55                  60

Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu
65                  70                  75                  80

Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr
                85                  90                  95

Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu
            100                 105                 110

Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly
        115                 120                 125

Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val
    130                 135                 140

Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val
145                 150                 155                 160

Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe
                165                 170                 175

Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile
            180                 185                 190

Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr
```

```
          195                 200                 205

Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu
    210                 215                 220

Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser
                245                 250                 255

Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val
            260                 265                 270

Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly
        275                 280                 285

Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met
    290                 295                 300

Leu Pro Glu Phe Gly Gln Leu Thr Ser Leu Ser Phe Asp Pro Gln Gly
305                 310                 315                 320

Gly Leu Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr
                325                 330                 335

Lys Arg Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr
            340                 345                 350

Val Phe Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile
        355                 360                 365

Cys Phe Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu
    370                 375                 380

Arg Asn Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu
385                 390                 395                 400

Val Asn Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile
                405                 410                 415

Pro Ser Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu
            420                 425                 430

Glu Glu Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly
        435                 440                 445

Gly Gly Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro
    450                 455                 460

Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val
465                 470                 475                 480

Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser
                485                 490                 495

Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu
            500                 505                 510
```

Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser
515 520 525

Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val
530 535 540

Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys
545 550 555 560

Thr Val

<210> IAS MBP 90-101 CH1.H
<211> 556
<212> PRT
<213> murine

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
5 10 15

Gly Ser Thr Gly Asp Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His
20 25 30

Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser
35 40 45

Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn
50 55 60

Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu
65 70 75 80

Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr
85 90 95

Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu
100 105 110

Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly
115 120 125

Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val
130 135 140

Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val
145 150 155 160

Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe
165 170 175

Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile
180 185 190

Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr
195 200 205

Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu
210 215 220

```
Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser
                245                 250                 255

Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val
            260                 265                 270

Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly
        275                 280                 285

Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met
    290                 295                 300

Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu
305                 310                 315                 320

Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg
                325                 330                 335

Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe
            340                 345                 350

Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe
        355                 360                 365

Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn
    370                 375                 380

Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn
385                 390                 395                 400

Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser
                405                 410                 415

Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu
            420                 425                 430

Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly
        435                 440                 445

Gly Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu
    450                 455                 460

Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe
465                 470                 475                 480

Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg
                485                 490                 495

Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser
            500                 505                 510

Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu
        515                 520                 525

Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser
```

530 535 540

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
545 550 555

<210> IAS MBP 1-14 CK
<211> 775
<212> PRT
<213> murine

Thr Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val
5 10 15

Pro Gly Ser Thr Gly Asp Met Ala Ser Gln Lys Arg Pro Ser Gln Arg
20 25 30

His Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp
35 40 45

Ser Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr
50 55 60

Asn Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg
65 70 75 80

Glu Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val
85 90 95

Thr Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu
100 105 110

Glu Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu
115 120 125

Gly Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val
130 135 140

Val Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu
145 150 155 160

Val Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp
165 170 175

Phe Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu
180 185 190

Ile Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met
195 200 205

Thr Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser
210 215 220

Leu Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser
225 230 235 240

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser
245 250 255

```
Ser Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr
            260                 265                 270

Val Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp
        275                 280                 285

Gly Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp
    290                 295                 300

Met Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly
305                 310                 315                 320

Leu Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys
                325                 330                 335

Arg Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val
            340                 345                 350

Phe Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys
        355                 360                 365

Phe Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg
    370                 375                 380

Asn Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val
385                 390                 395                 400

Asn Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro
                405                 410                 415

Ser Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu
            420                 425                 430

Glu Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly
        435                 440                 445

Gly Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly
    450                 455                 460

Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys
465                 470                 475                 480

Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu
                485                 490                 495

Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr
            500                 505                 510

Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu
        515                 520                 525

Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp
    530                 535                 540

Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr
545                 550                 555                 560

Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp
                565                 570                 575
```

Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp
580 585 590

Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp
595 600 605

Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn
610 615 620

Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp
625 630 635 640

Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro
645 650 655

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala
660 665 670

Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp
675 680 685

Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile
690 695 700

Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn
705 710 715 720

Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys
725 730 735

Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys
740 745 750

Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu
755 760 765

Ser His Ser Pro Gly Lys
770

<210> IAS MBP 1-14 CH1.H.CH2
<211> 561
<212> PRT
<213> murine

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
5 10 15

Gly Ser Thr Gly Asp Met Ala Ser Gln Lys Arg Pro Ser Gln Arg His
20 25 30

Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser
35 40 45

Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn
50 55 60

Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu

65 70 75 80

Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr
85 90 95

Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu
100 105 110

Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly
115 120 125

Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val
130 135 140

Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val
145 150 155 160

Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe
165 170 175

Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile
180 185 190

Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr
195 200 205

Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu
210 215 220

Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly
225 230 235 240

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser
245 250 255

Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val
260 265 270

Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly
275 280 285

Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met
290 295 300

Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu
305 310 315 320

Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg
325 330 335

Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe
340 345 350

Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe
355 360 365

Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn
370 375 380

Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn
385 390 395 400

Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser
405 410 415

Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu
420 425 430

Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly
435 440 445

Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
450 455 460

Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly
465 470 475 480

Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser
485 490 495

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr
500 505 510

Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr
515 520 525

Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys
530 535 540

Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val
545 550 555 560

<210> IAS MBP 90-101 CH1.H.CH2.CH3
<211> 773
<212> PRT
<213> murine

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
5 10 15

Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro
20 25 30

Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg
35 40 45

His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr
50 55 60

Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr
65 70 75 80

Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu
85 90 95

Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr
100 105 110

```
Arg Ala Glu Leu Asp Thr Thr Cys Arg His Asn Tyr Glu Gly Val Glu
        115                 120                 125

Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val Ile Ser
    130                 135                 140

Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val Cys Ser
145                 150                 155                 160

Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe Arg Asn
                165                 170                 175

Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile Arg Asn
            180                 185                 190

Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr Pro Arg
        195                 200                 205

Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu Lys Ser
    210                 215                 220

Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
225                 230                 235                 240

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Glu Asp
                245                 250                 255

Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln
            260                 265                 270

Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu
        275                 280                 285

Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro
    290                 295                 300

Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn
305                 310                 315                 320

Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn
                325                 330                 335

Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe Pro Lys
            340                 345                 350

Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe Val Asp
        355                 360                 365

Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys
    370                 375                 380

Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp
385                 390                 395                 400

His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp
                405                 410                 415

Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val
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                420                 425                 430

Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Leu
        435                 440                 445

Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala
    450                 455                 460

Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr
465                 470                 475                 480

Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser
                485                 490                 495

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu
            500                 505                 510

Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val
        515                 520                 525

Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys
    530                 535                 540

Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
545                 550                 555                 560

Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
                565                 570                 575

Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
            580                 585                 590

Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
        595                 600                 605

Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
    610                 615                 620

Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
625                 630                 635                 640

Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
                645                 650                 655

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
            660                 665                 670

Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
        675                 680                 685

Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
    690                 695                 700

Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
705                 710                 715                 720

Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
                725                 730                 735
```

Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
740 745 750

Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
755 760 765

Ser Pro Gly Lys
770

WHAT IS CLAIMED IS:

1. A multimeric complex comprising a first single chain MHC class II heterodimer and a second single chain MHC class II heterodimer, wherein the first and the second single chain MHC class II heterodimers each comprise an α1 domain and a β1 domain linked via an amino acid linker and a multimerization domain, and wherein the first and the second single chain MHC class II heterodimers are linked via the multimerization domain to form a multimeric complex.

2. The multimeric complex of claim 1, wherein the first and the second single chain MHC class II heterodimers are each linked to an antigenic peptide via an amino acid linker.

3. The multimeric complex of claim 2, wherein the peptides are the same.

4. The multimeric complex of claim 2, wherein the peptides are from the same antigen.

5. The multimeric complex of claim 2, wherein the peptides are from different antigens.

6. The multimeric complex of claim 2, wherein the peptides are selected from the group consisting of MBP83-102Y83, PLP 40-60, PLP 89-106, PLP 95-117, and PLP 185-206.

7. The multimeric complex of claim 1, wherein the first and the second MHC class II heterodimers are from the same MHC class II allele.

8. The multimeric complex of claim 1, wherein the first and the second MHC class II heterodimers are from different MHC class II alleles.

9. The multimeric complex of claim 7 or 8, wherein the alleles are selected from the group consisting of DRB1*1501 and DRB5*0101.

10. The multimeric complex of claim 7 or 8, wherein the first and the second single chain MHC class II heterodimers are each linked to an antigenic peptide via an amino acid linker.

11. The multimeric complex of claim 10, wherein the peptides are the same.

12. The multimeric complex of claim 10, wherein the peptides are from the same antigen.

13. The multimeric complex of claim 10, wherein the peptides are from different antigens.

14. The multimeric complex of claim 10, wherein the peptides are selected from the group consisting of MBP83-102Y83, PLP 40-60, PLP 89-106, PLP 95-117, and PLP 185-206.

15. The multimeric complex of claim 1, wherein the first and second MHC class II heterodimers are from a human.

16. The multimeric complex of claim 1, wherein the multimerization domains are covalently linked.

17. The multimeric complex of claim 1, wherein the multimerization domains are non-covalently linked.

18. The multimeric complex of claim 1, wherein the multimerization domain is a leucine zipper domain.

19. The multimeric complex of claim 1, wherein the multimerization domain is an immunoglobulin domain.

20. The multimeric complex of claim 19, wherein the immunoglobulin domain is a Cκ domain or a heavy chain domain.

21. The multimeric complex of claim 1, wherein the first or the second MHC class II heterodimer is selected from the group consisting of I-As MBP.β1β2α1α2.Cκ, I-As MBP.β1β2α1α2.CH1.H, I-As MBP.β1β2α1α2.CH1.H.CH2, and I-As MBP.β1β2α1α2.CH1.H.CH2.CH3.

22. The multimeric complex of claim 1, further comprising a third and a fourth MHC class II heterodimer, wherein the third and the fourth MHC class II heterodimer each comprise an α1 domain and a β1 domain linked via an amino acid linker and a multimerization domain, and wherein the first, second, third and fourth single chain MHC class II heterodimers are linked via the multimerization domain to form a multimeric complex.

23. The multimeric complex of claim 1, wherein the first and the second MHC class II heterodimers comprise β1 β2 domains and α1 α2 domains linked via an amino acid linker.

24. The multimeric complex of claim 1 or 2, wherein the linker is selected from the group consisting of GG, GGGG, GGGGS, GGGSG, ASGGGSGGG, TSGGGGSGGGGSSS, GSPGGGGSGGGPGS, GSPPGGPPGS, GSPGGGGPGS, TSGGGGS, SGGSGGS, FDAPSPLP, and VYPEVTV.

25. The multimeric complex of claim 1 or 2, wherein the linker is from a CD4 molecule.

26. The multimeric complex of claim 25, wherein the linker is selected from the group consisting of RNGQEEKAGVVSTGLI ,RNGQETKAGVVSTGLI, YNQQEEKAGGVSTGLI, FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI, FYNQQEEKAGGVSTGLI, and LNGQEEKAGMVSTGLI.

27. The multimeric complex of claim 1, wherein the first or the second MHC class II heterodimer is selected from the group consisting of CO523, CO543, CO563, CO567, CO580, CO581, CO582, CO583, CO584, CO585, CO586, CO587, CO588, CO589, CO590, CO591, CO592, CO593, CO594, CO595, CO596, CO597, and CO608.

28. The multimeric complex of claim 1, wherein the first MHC class II or the second MHC class II heterodimer is encoded by a nucleic acid that codons optimized for prokaryotic expression.

29. The multimeric complex of claim 28, wherein the prokaryote is *E. coli.*

30. The multimeric complex of claim 28, wherein the nucleic acid encoding the first or the second MHC class II heterodimer is selected from the group consisting of CO528-AC and CO608-AC.

31. A pharmaceutical composition comprising the multimeric complex of claim 2.

32. The pharmaceutical composition of claim 31, further comprising an adjuvant.

33. A method of treating autoimmune disease in a subject, the method comprising administering an immunogenically effective amount of a pharmaceutical composition comprising the multimeric complex of claim 2.

34. The method of claim 33, wherein the subject is a human.

35. A recombinant nucleic acid encoding a single chain MHC class II heterodimer comprising an α1 domain and a β1 domain linked via an amino acid linker, wherein the linker is selected from the group consisting of GG, GGGG, GGGGS, GGGSG, ASGGGSGGG, TSGGGGSGGGGSSS, GSPGGGGSGGGPGS, GSPPGGPPGS, GSPGGGGPGS, TSGGGGS, SGGSGGS, FDAPSPLP, and VYPEVTV.

36. The nucleic acid of claim 35, wherein the MHC class II heterodimer is selected from the group consisting of CO523, CO543, CO563, CO567, CO580, CO581, CO582, CO583, CO584, CO585, CO586, CO587, CO588, CO589, CO590, CO591, CO592, CO593, CO594, CO595, CO596, CO597, and CO608.

37. A recombinant nucleic acid encoding a single chain MHC class II heterodimer comprising an α1 domain and a β1 domain linked via an amino acid linker, wherein the linker is wherein the linker is from a CD4 molecule.

38. The nucleic acid of claim 37, wherein the linker is selected from the group consisting of RNGQEEKAGVVSTGLI ,RNGQETKAGVVSTGLI, YNQQEEKAGGVSTGLI, FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI, FYNQQEEKAGGVSTGLI, and LNGQEEKAGMVSTGLI.

39. A recombinant nucleic acid encoding a single chain MHC class II heterodimer selected from the group consisting of CO528-AC and CO608-AC.

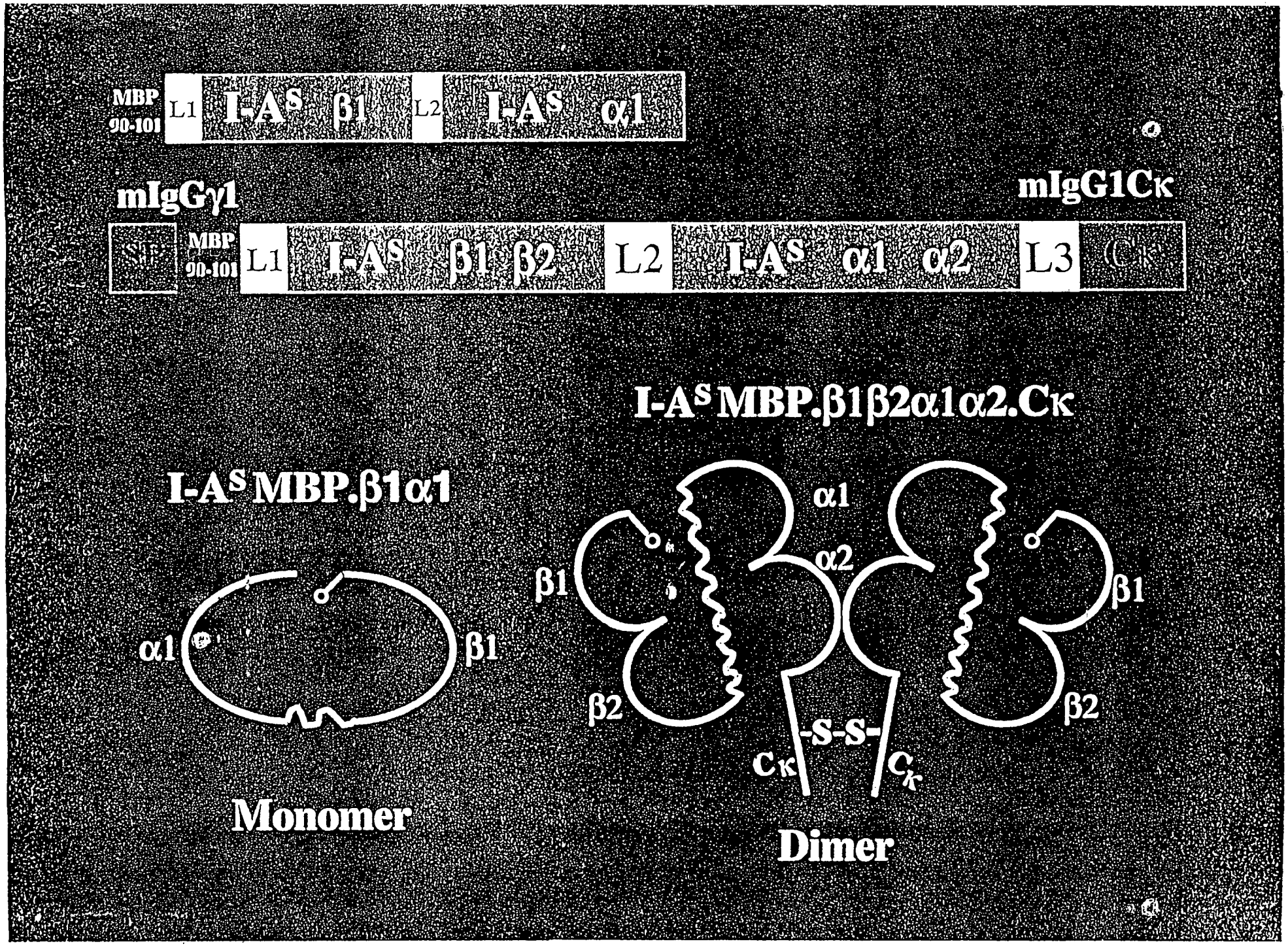
40. A recombinant nucleic acid encoding a single chain MHC class II heterodimer selected from the group consisting I-As MBP.β1β2α1α2.Cκ, I-As MBP.β1β2α1α2.CH1.H, I-As MBP.β1β2α1α2.CH1.H.CH2, and I-As MBP.β1β2α1α2.CH1.H.CH2.CH3.

IMMUNE MEDIATORS AND RELATED METHODS

ABSTRACT OF THE INVENTION

The present invention relates to nucleic acids encoding single chain MHC class II molecules that form multimers via inter-chain multimerization domains, and methods of treating autoimmune disease using the same.

SF 1139077 v1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L1
I-A^S β1
L2
I-A^S α1
mIgGγ1
mIgG1Cκ
SP
MBP 90-101
L1
I-A^S β1 β2
L2
I-A^S α1 α2
L3
(Cκ)
I-A^{S}MBP.β1α1
α1
β1
Monomer
I-A^{S}MBP.β1β2α1α2.Cκ
α1
α2
β1
β1
β2
β2
-s-s-
Cκ
Cκ
Dimer

Figure 1

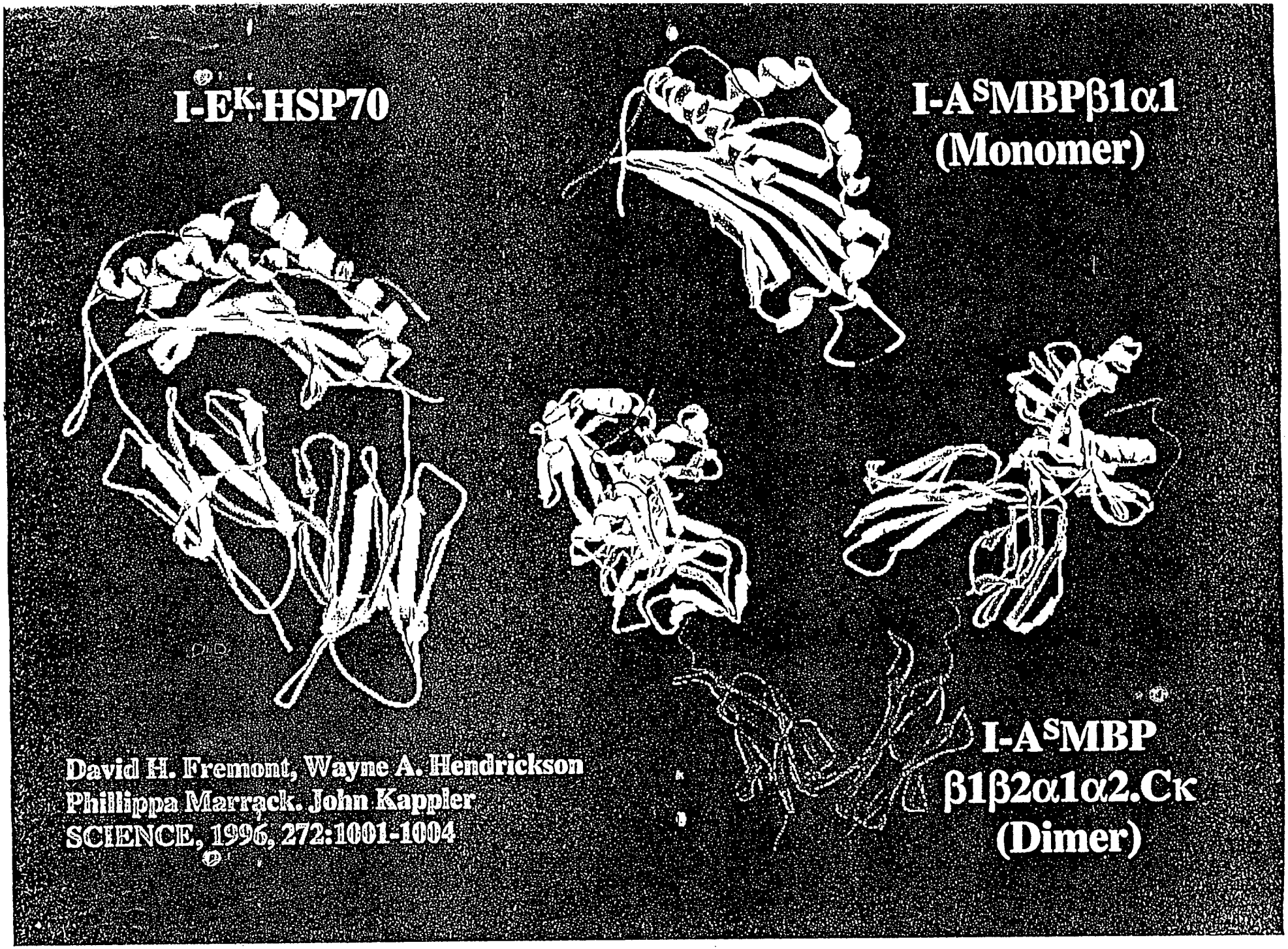

I-E^{K}:HSP70
I-A^{S}MBPβ1α1
(Monomer)
David H. Fremont, Wayne A. Hendrickson
Phillippa Marrack, John Kappler
SCIENCE, 1996, 272:1001-1004
I-A^{S}MBP
β1β2α1α2.Cκ
(Dimer)

Figure 2

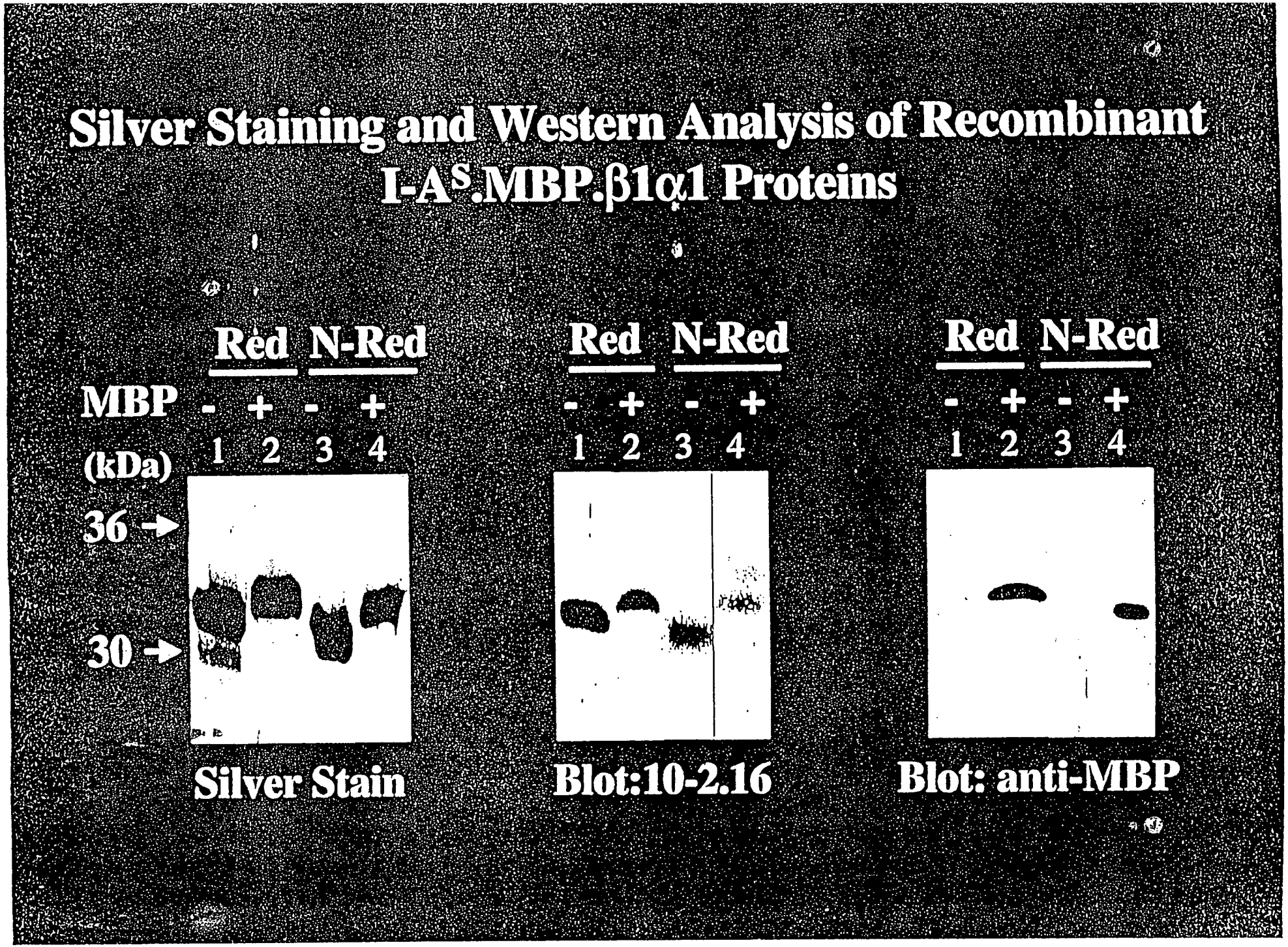

Silver Staining and Western Analysis of Recombinant I-A^S.MBP.β1α1 Proteins
Red N-Red
Red N-Red
Red N-Red
MBP - + - +
MBP - + - +
MBP - + - +
1 2 3 4
1 2 3 4
1 2 3 4
(kDa)
36
30
Silver Stain
Blot:10-2.16
Blot: anti-MBP

Figure 3

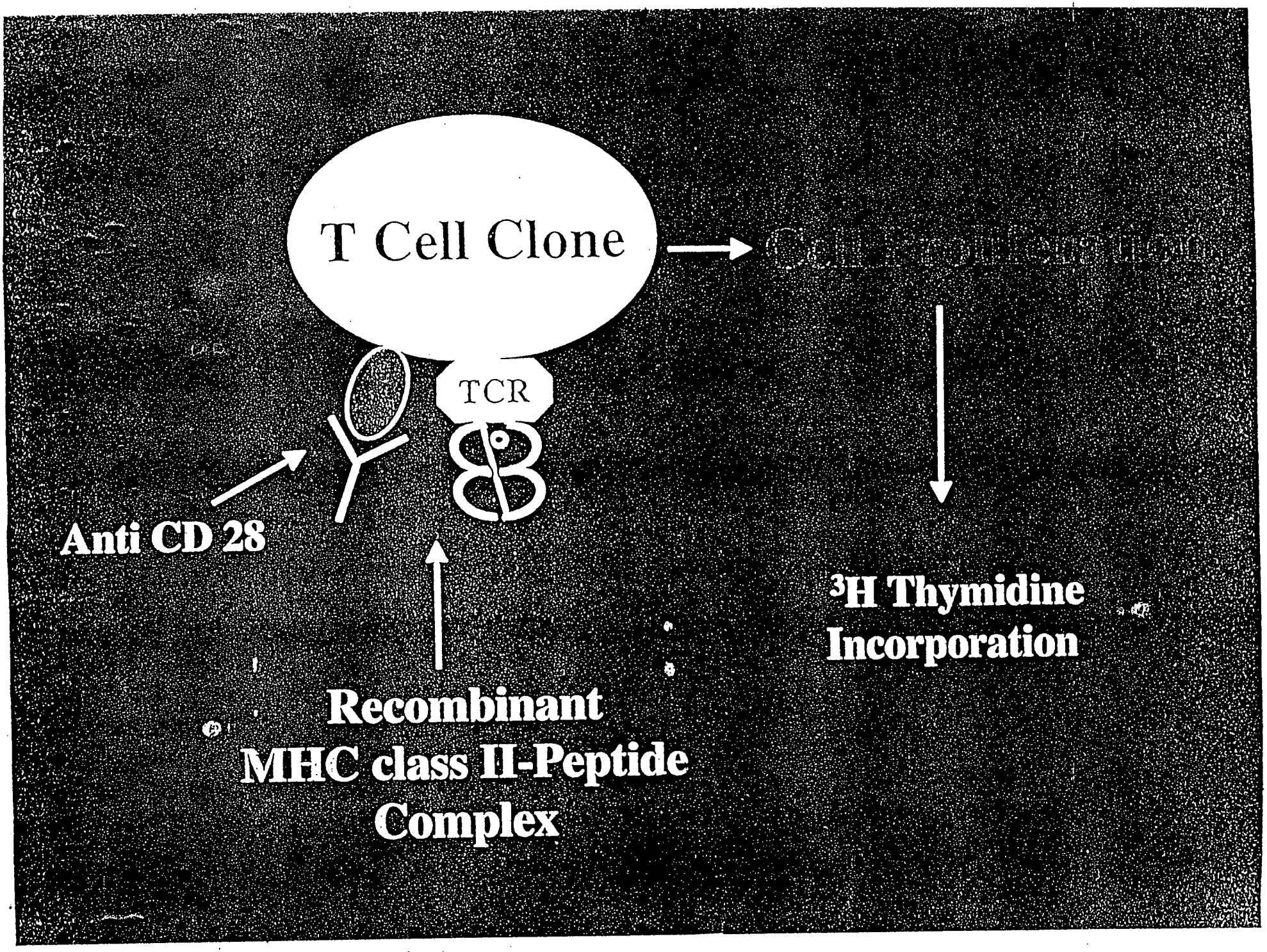

T Cell Clone
TCR
Anti CD 28
Recombinant
MHC class II-Peptide
Complex
^{3}H Thymidine
Incorporation

Figure 4

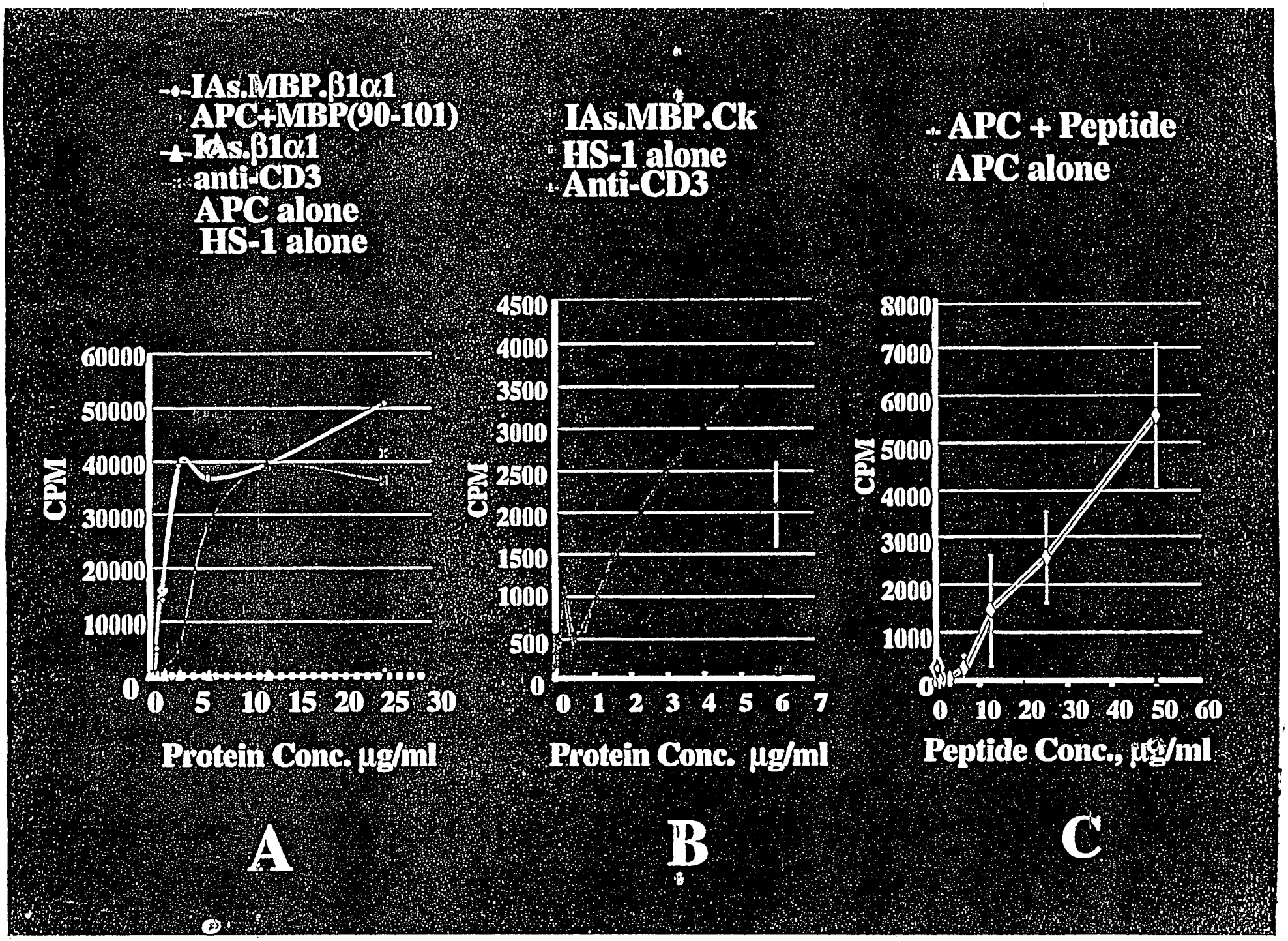

IAs.MBP.β1α1
APC+MBP(90-101)
IAs.β1α1
anti-CD3
APC alone
HS-1 alone
IAs.MBP.Ck
HS-1 alone
Anti-CD3
APC + Peptide
APC alone
CPM
60000
50000
40000
30000
20000
10000
0
0 5 10 15 20 25 30
Protein Conc. µg/ml
A
CPM
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 1 2 3 4 5 6 7
Protein Conc. µg/ml
B
CPM
8000
7000
6000
5000
4000
3000
2000
1000
0
0 10 20 30 40 50 60
Peptide Conc., µg/ml
C

Figure 5

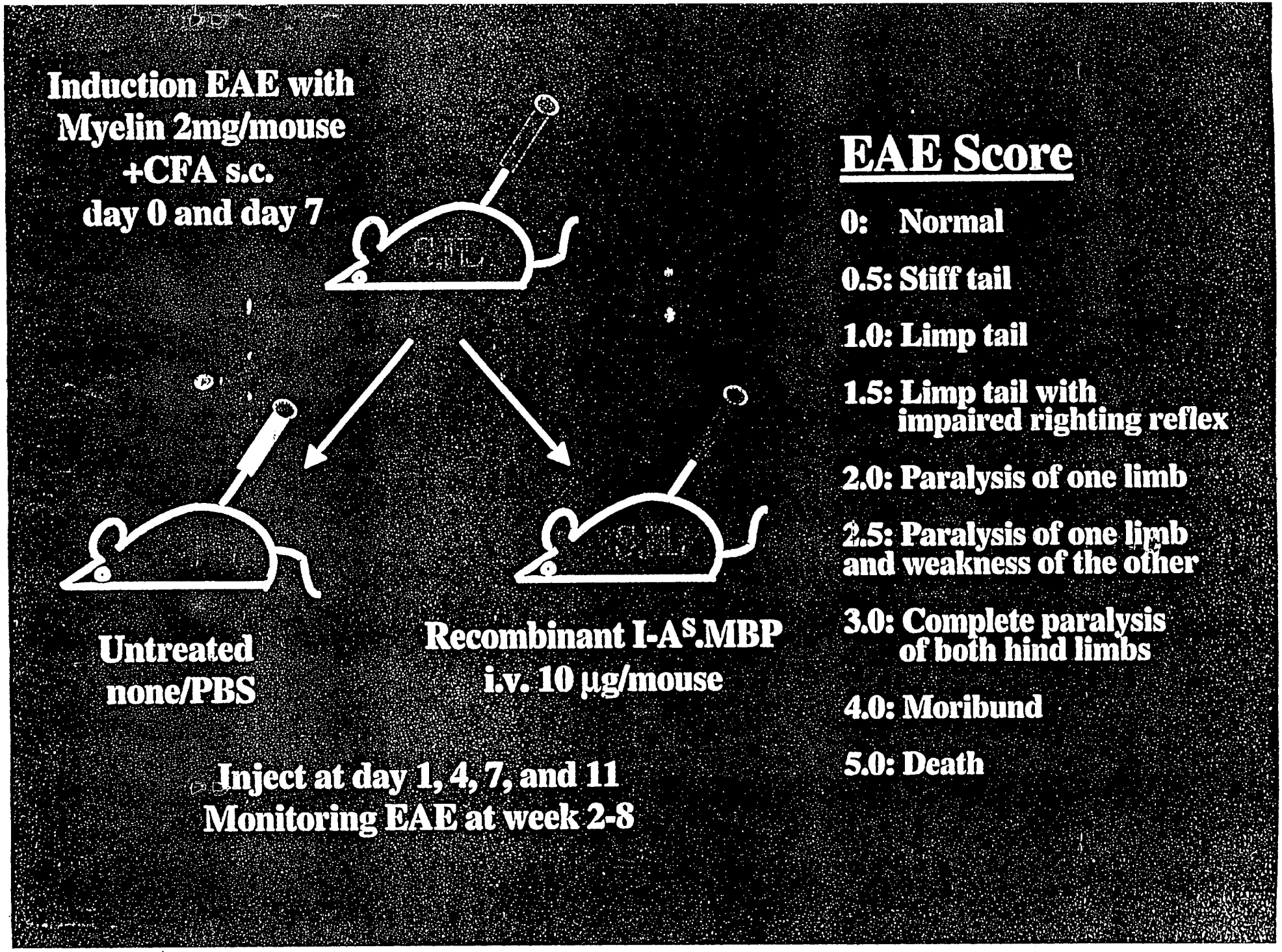

Induction EAE with
Myelin 2mg/mouse
+CFA s.c.
day 0 and day 7
EAE Score
0: Normal
0.5: Stiff tail
1.0: Limp tail
1.5: Limp tail with impaired righting reflex
2.0: Paralysis of one limb
2.5: Paralysis of one limb and weakness of the other
3.0: Complete paralysis of both hind limbs
4.0: Moribund
5.0: Death
Untreated
none/PBS
Recombinant I-A^{s}.MBP
i.v. 10 µg/mouse
Inject at day 1, 4, 7, and 11
Monitoring EAE at week 2-8

Figure 6

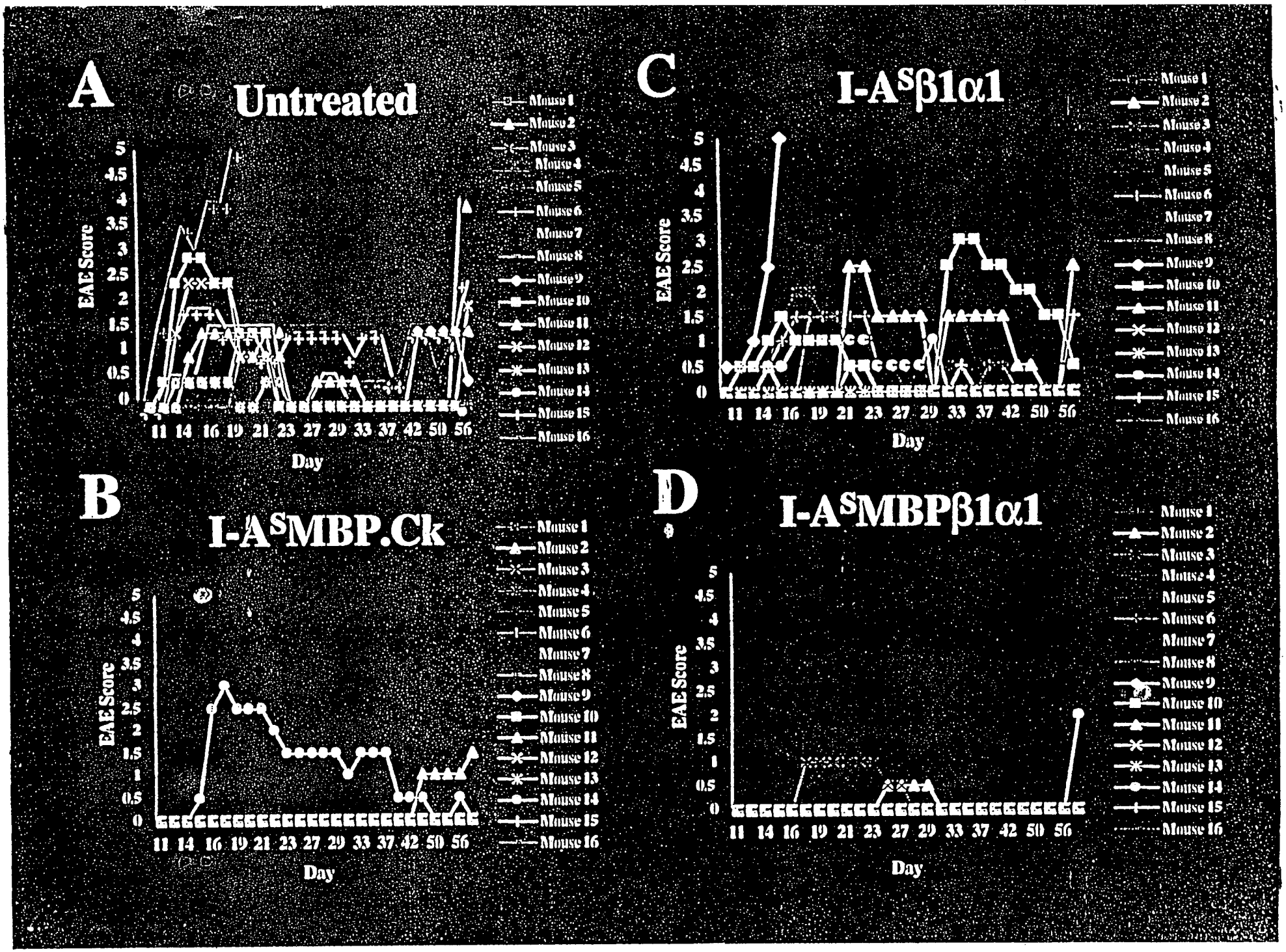

A
Untreated
EAE Score
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Mouse 9
Mouse 10
Mouse 11
Mouse 12
Mouse 13
Mouse 14
Mouse 15
Mouse 16
C
I-A^{S}β1α1
B
I-A^{S}MBP.Ck
D
I-A^{S}MBPβ1α1

Figure 7

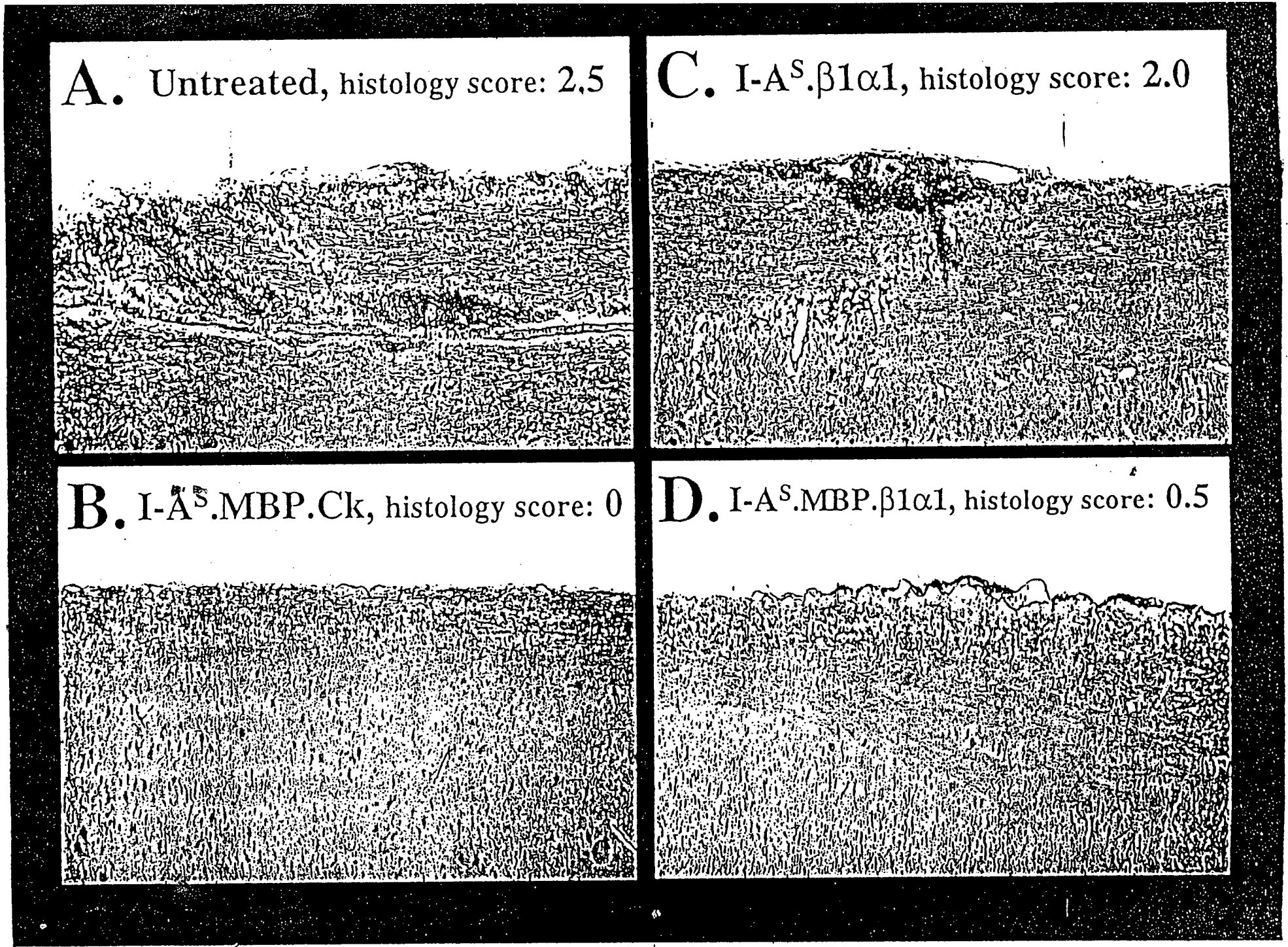
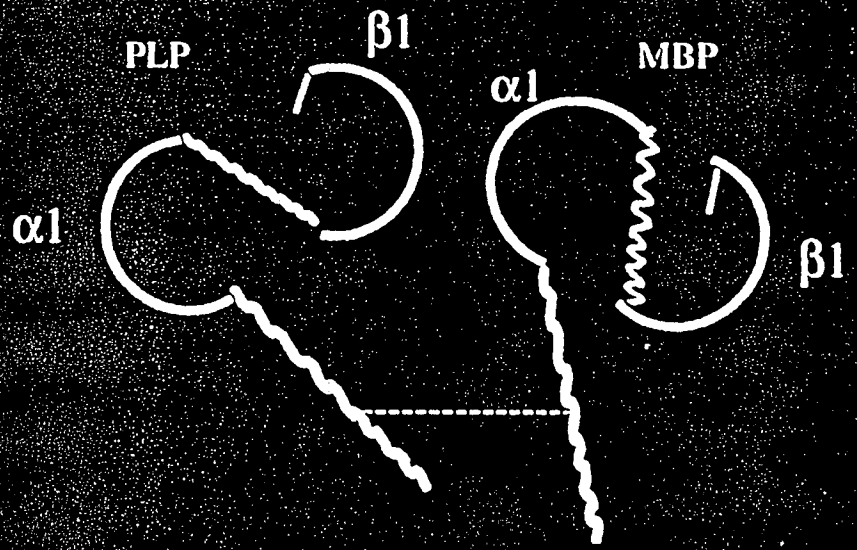

A. Untreated, histology score: 2.5
C. I-A^S.β1α1, histology score: 2.0
B. I-A^S.MBP.Ck, histology score: 0
D. I-A^S.MBP.β1α1, histology score: 0.5

Figure 8

Schematic representation of heterodimeric trucncated MHC class II-peptide molecules with two different peptide specificities.



The dimerization can be achieved by the use site specific chemical conjugation or by the use of recombinant methods by the co-expression of MHC-Peptide molecules with different specificities having dimer forming amino acid tails (example: Ck, Leucine zipper)

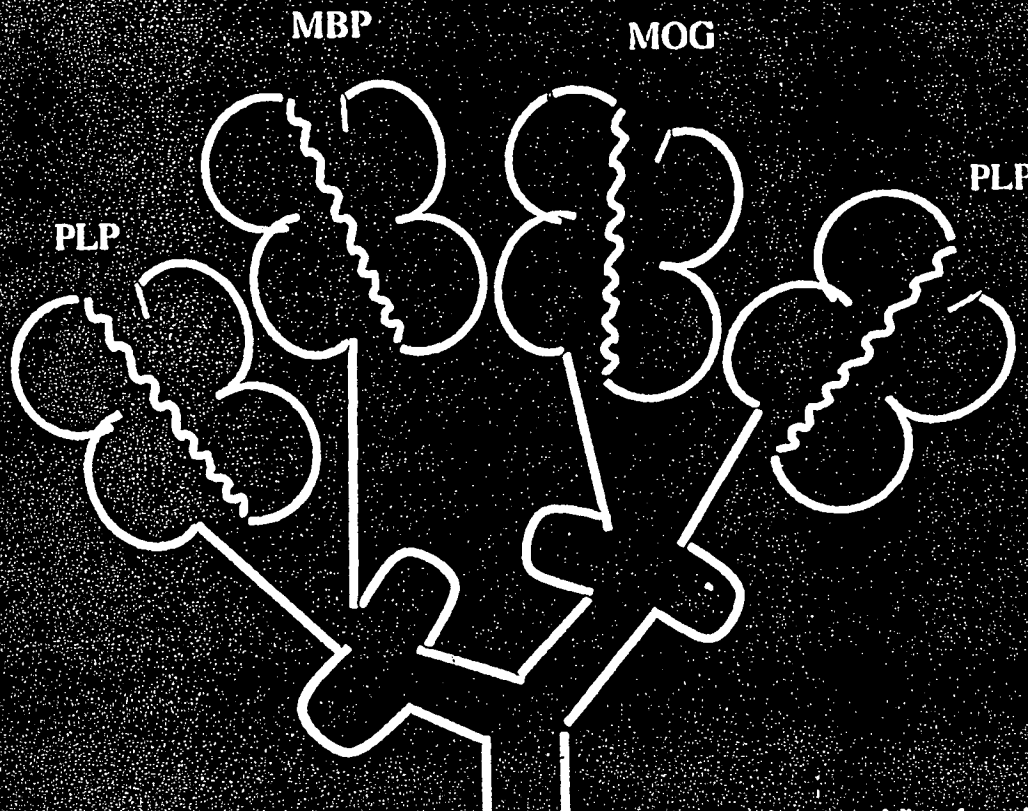
Figure 9

Schematic representation of MHC-Peptide Heterodimer

The dimerization can be achieved by chemical conjugation of two different MHC-Peptide molecules or can be achieved by the use of recombinant methods by the co-expression MHC-Peptide molecules containing dimer forming amino acid sequences (example: Lucine zipper, Ck, IgG heavy chain and light chain etc)

Figure 10

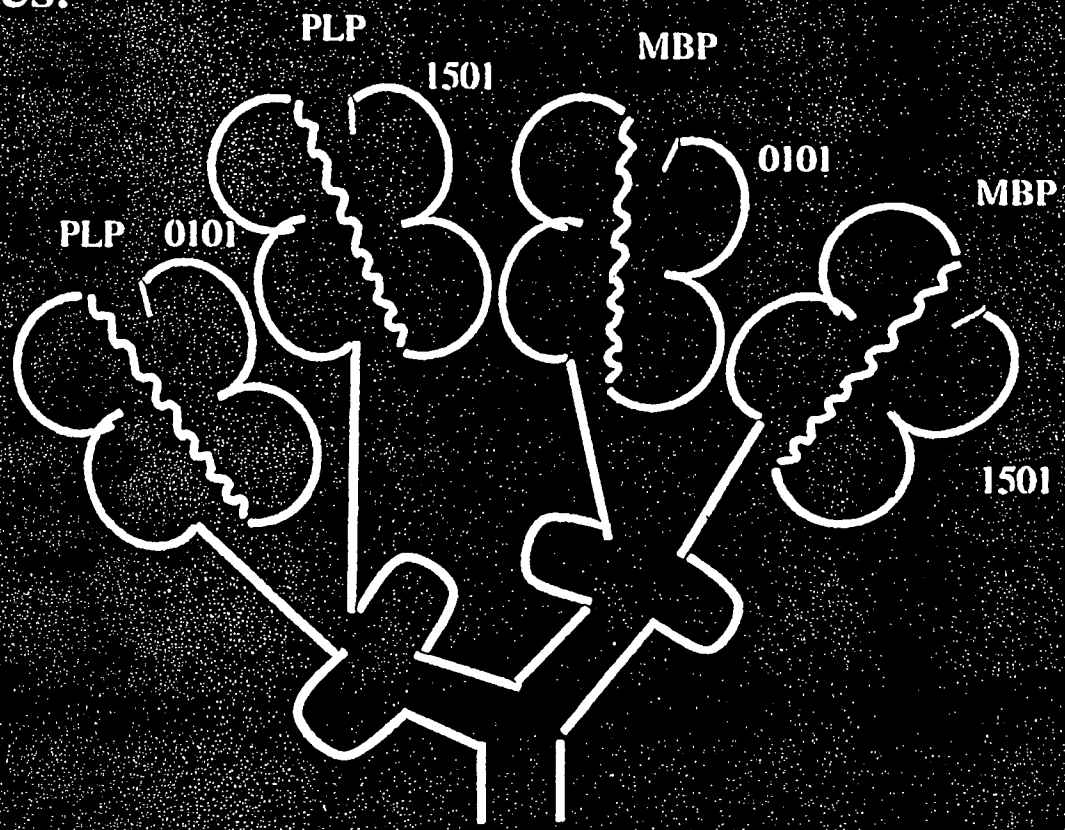
Schematic representation of MHC-Peptide hetero-tetramer with binding capability to four different antigen specific T cells.



The tetramerization of MHC-peptide molecules can be achieved by site specific chemical conjugation of four different MHCpeptide complexes or by recombinant methods. The recombinant methods includethe appropriate use of IgG heavy and light chain tails to first express the heterodimer and then form the tetramer in vitro.

Figure 11

Schematic representation tetrameric MHC-Peptide complexes containing two different alleles of MHC class II and two different peptides.



DRB1*1501 & DRB5*0101 continuation with PLP & MBP & MOG peptides

Figure 12

Figure 13

Effect of different recombinant MHC class II molecules on the development of EAE (day +60)

EAE induction Day 0 & 7	Treatment Day 1,4, 7 & 11 10 ug/injection i.v.	Incidence of disease (%)
Myelin 2 mg/mouse in CFA s.c.	none	12/16 (75%)
Myelin 2 mg/mouse in CFA s.c.	Monomer	2/12 (16 %)
Myelin 2 mg/mouse in CFA s.c.	Dimer	2/6 (33%)
Myelin 2 mg/mouse in CFA s.c.	Tetramer	3/14 (21%)
Myelin 2 mg/mouse in CFA s.c.	Monomer Pool	2/16 (12%)
Myelin 2 mg/mouse in CFA s.c.	Half Anergix	2/16 (12%)
Myelin 2 mg/mouse in CFA s.c.	I-As peptide	9/16 (56%)
Myelin 2 mg/mouse in CFA s.c.	Empty Half Anergix	10/16 (62%)